

Role of C-reactive protein and interleukin-6 in the diagnosis of neonatal sepsis

Introduction:

Neonatal sepsis is a major cause of and mortalities in developing countries. Early diagnosis and treatment of the newborn infant with suspected sepsis are essential to prevent severe and life threatening complications. Early detection of bacterial sepsis is difficult for various reasons: firstly, early warning signs and symptoms are often non-specific. Then there is the difficulty of distinguishing the clinical picture of neonatal sepsis from non-infectious causes. Further, microbiological culture results are not usually available until at least 48-72 hours after the specimen reaches the laboratory, and high false-negative rates of culture results may occur.

Aims and objectives:

1. Bacteriological profile of neonatal septicemia and antibiotic susceptibility pattern of the isolates.
2. To determine the value of C-reactive protein and Interleukin-6 in establishing the diagnosis of neonatal sepsis.

Material and Methods:

Sample size: 100 cases of clinically suspected sepsis cases admitted in Neonatal Intensive Care Unit, Tirunelveli Medical College Hospital.

Blood samples will be collected from all clinically suspected sepsis cases. The samples will be collected under aseptic precaution and all the samples will be analyzed by routine blood culture and serum used for detection of C-reactive protein and interleukin-6.

Results:

A total of 100 neonates (0 to 28 days) who fulfilled the criteria of clinically suspected sepsis were analyzed. Out of 100 study cases, 26 cases were blood culture positive. *Klebsiella spp* were the most common organisms for causing both EONS and LONS in the present study.

Serum CRP level detected by latex agglutination method had 53.84% of sensitivity and 64.86% of specificity against blood culture as a reference test.

Serum IL-6 level detected by ELISA had sensitivity of 96.15% and specificity of 81.08% against blood culture as a reference test. Positive predictive value and negative predictive value of IL-6 were 64.1% and 98.36% respectively.

Conclusion:

The findings of the present study confirm that the serum level of IL-6 is a more reliable marker than the serum levels of CRP. Serum level of IL-6 is also useful in evaluation of response of neonatal sepsis to antibiotic therapy.

Keywords: Interleukin-6, C-reactive protein, neonate, sepsis, antimicrobial susceptibility.

1. INTRODUCTION

Neonatal sepsis is a clinical syndrome characterized by systemic signs of infection, accompanied by bacteremia within the first four weeks of life (28 days). Neonatal sepsis is the most common cause of morbidity and mortality in neonatal period.¹ Every year 135 million babies are born alive worldwide. Statistical data in 2011 estimated 3.0 million of these died during the first four weeks of life.¹

Neonatal sepsis is classified into early onset neonatal sepsis (EONS) and late onset neonatal sepsis (LONS) according to time of onset of signs and symptoms. Early onset neonatal sepsis is defined as the onset of signs and symptoms within the first 72 hours of life.² In late onset neonatal sepsis (LONS) clinical signs and symptoms occurs after 72 hours of age.³

Early Onset Neonatal Sepsis, which occurs within the first 72 hours of life, usually presents with respiratory distress and pneumonia. In severe cases, the neonate may be symptomatic at birth.

Infection may be acquired through the transplacental route during in utero period or transcervical route during birth. Ascending infection through the cervix, with or without rupture of the amniotic fluid membranes may result in amnionitis, funisitis (infection of the umbilical cord), congenital pneumonia and sepsis.⁴

Late Onset Neonatal Sepsis usually presents after 72 hours of age and can either be nosocomial (hospital-acquired) or community-acquired infections. The most common

cause of late onset sepsis is nosocomial infection from neonatal intensive care unit. Preterm babies and low birth weight infants are mainly affected. The risk factors for late onset sepsis are prematurity, low birth weight, male sex, low serum Ig G levels, low Apgar scores, mechanical ventilation, prolonged use of intravascular catheters, total parenteral nutrition and delayed enteral feedings. These neonates are mainly diagnosed to have septicemia, pneumonia or meningitis.⁵

Early diagnosis and prompt and appropriate treatment of all neonates with clinical suspicion of sepsis has been found to be an important strategy in preventing neonatal mortality and life threatening complications.

Initial diagnosis of neonatal sepsis based on clinical signs and symptoms which are non-specific as other non-infective condition like aspiration, asphyxia and metabolic disorders may also present with similar signs mimicking sepsis. The problem of symptom wise false positivity in diagnosing sepsis resulting in unwarranted initiation of empirical antibiotic therapy may lead to development of drug resistance, prolonged hospital stay, increased treatment cost and the separation of the neonates from their mothers.

Diagnosis of neonatal sepsis is broadly classified into direct method and indirect methods.

Direct method: Isolation of causative microorganisms of sepsis from blood, CSF, urine, pleural fluid or pus is diagnostic.

Indirect method: Over the last decade, a variety of laboratory tests have been developed

to enhance the early and accurate identification and treatment of neonates with suspected sepsis. Those are haematological markers, serological sepsis markers and radiological evidences.

The gold standard method for diagnosis of neonatal sepsis is isolation of microorganism from blood, CSF, urine and pleural fluid. It is time consuming procedure usually takes more than three days for complete result and also requires well equipped laboratory and trained personnel for better results. Hence alternative fast diagnostic test of serological markers enabling earlier detection of neonatal sepsis might be beneficial.

Increased neonatal mortality in neonatal sepsis scenario necessitates need of rapid and effective diagnostic test with 100% sensitivity and 100% specificity. Such an ideal test is not available at this point of time and yet to be invented.

Hence combinations of clinical signs, haematological and serological markers have been proved to be a useful strategy in the diagnosis of neonatal sepsis in resource poor settings.⁶ Commonly used diagnostic haematological markers such as total leucocyte count, immature: total neutrophil ratio, platelet count, absolute neutrophil count and micro erythrocyte sedimentation rate are less sensitive and specific for diagnosing neonatal sepsis.

In recent days screening of serological markers such as C-reactive protein (CRP), and various cytokines have been suggested as being useful and more sensitive indicators for early identification of sepsis in neonates. The biomarkers are classified into early

phase marker (Interleukin-6, Interleukin-8, Tumour Necrosis factor- α and Interferon- γ), mid phase marker (Procalcitonin) and late phase marker (C-reactive protein).⁷

C-reactive protein (CRP) is an acute-phase protein synthesized by the liver within six hours after the onset of inflammation and tissue necrosis. Its rapid synthesis, short half-life and rapid decline with recovery, together with its increase in serious bacterial infections, have made the CRP test popular in the diagnosis of infections.⁸ But it may also rise in systemic inflammatory conditions and giving rise to false positive results which limits its usage as specific diagnostic test.

Cytokines are main diagnostic markers and their levels are increased first in the infectious process. Interleukins are pro-inflammatory cytokines. Interleukins are produced by monocytes, activated macrophages and endothelial cells. C-reactive protein is induced by interleukin-6(IL-6).

Numerous studies have reported on the effectiveness of the quantitative measurement of IL-6 for an early diagnosis of neonatal sepsis.

Interleukin-6 and Tumour Necrosis Factor - α (TNF- α) are precursors to C-reactive protein in the inflammatory sequence. These serological markers rise early in infection. Hence evaluation of IL-6 alone or with CRP helps in early detection of infection and effective management of neonatal sepsis. Interleukin-6 is an early phase marker. C-reactive protein is a late phase marker. The purpose of this study is to detect the role of CRP and IL-6 in the diagnosis of neonatal sepsis.

2. AIMS AND OBJECTIVES

- ❖ To detect the bacteriological profile of neonatal sepsis and antibiotic susceptibility pattern of the isolates in Tirunelveli Medical College.
- ❖ To determine the value of C-reactive protein and Interleukin-6 in establishing the diagnosis of neonatal sepsis.
- ❖ To compare the efficacy of C-reactive protein and Interleukin-6 with conventional blood culture method for the diagnosis of neonatal sepsis.

3. REVIEW OF LITERATURE

Neonatal sepsis is a clinical syndrome characterized by systemic signs of infection and isolation of a pathogen from blood, cerebral spinal fluid and from any other sterile site within the first 28 days of life. Bacterial infection is a main cause of neonatal sepsis.

It includes various systemic infections of the newborn such as septicemia, meningitis, pneumonia, arthritis, osteomyelitis, and urinary tract infections. Superficial infections like conjunctivitis and oral thrush are not included under neonatal sepsis.⁹

In developed countries the mortality from neonatal sepsis is found to be in declining trend due to improved health care and appropriate use of antibiotics. But, the mortality due to neonatal sepsis is still high in developing countries accounting up to 50% of neonatal deaths.¹⁰

Rapid diagnosis and appropriate treatment is very essential in reducing morbidity and mortality associated with neonatal sepsis.¹¹ The empiric use of antimicrobial treatment to all neonates presenting with clinical symptoms of sepsis practiced in resource poor settings in developing countries exposes neonates to adverse drug effects and promotes the development of drug resistant strains.⁴

3.1 Classification of neonatal sepsis

Neonatal sepsis is classified into early onset neonatal sepsis and late onset neonatal sepsis according to time of onset of signs and symptoms.

3.1.1 Risk factors for early onset neonatal sepsis are¹²

- Low birth weight – less than 2500 grams or prematurity
- Febrile illness in the mother with evidence of bacterial infection within two weeks prior to delivery.
- Foul smelling liquor and / or meconium stained liquor.
- Prolonged rupture of membranes - more than 24 hours.
- More than three vaginal examinations during labour.
- Prolonged labour – more than 24 hours.
- Perinatal asphyxia.

3.1.2 Risk factors for late onset neonatal sepsis are⁵

- Low birth weight – less than 2500 grams or prematurity.
- Low Apgar scores.
- Low Immuglobulin G (IgG) levels.
- Prolonged use of intravascular catheters.
- Treatment with steroids.
- Total parenteral nutrition.
- Delayed enteral feedings.
- Male sex.

The incidences of neonatal sepsis are more common in male infants compared to female infants. This male predominance may be due to X-linked immunoregulatory gene factor contributing to increased host's susceptibility to infections in male neonates.

A study conducted in Nepal during period of two years from July 2007 to June 2009 by YR Khinchi AK *et al*, found that among 175 neonatal sepsis cases, 65.1% were male infants and 34.9% were female infants.¹³

Based on the study conducted by Rekha Sriram *et al*, Sri Devaraj Urs Medical College, Karnataka, India, out of 115 clinically suspected sepsis cases, 76 (66.1%) were males and 39 (33.9%) were females. In these 48 cases were cultures positive. Among culture positive cases, male infants were 35 (60.3%) and female were 23 (39.7%). Male infants were predominately affected compared to female infants with a ratio of 1.5:1.¹⁴

A study conducted by Sucila Thangam *et al* found that among 50 clinically suspected neonatal cases, EONS cases were 58% and remaining 42% were under category of LONS.¹⁶

Another study from Tanzania, during March to November 2009 done by Neema Kayange *et al* reported that 60% cases were LONS and 40% cases were EONS among 300 clinically suspected neonatal sepsis cases.¹⁷

Recent study done by Flora Chacha *et al* at Catholic University of Health and Allied Sciences, Tanzania during October 2013 to April 2014 revealed among 305

clinically suspected sepsis cases, 224 cases (73.4%) were under the age group of 0-3 days (less than 72 hours).¹⁵

The incidence of neonatal sepsis is inversely associated to gestational age. A study conducted by Seo *et al.* revealed that increased sepsis incidence of 16.6% in preterm neonates with a gestational age less than 28 weeks and only 0.6% of incidence of neonatal sepsis in term neonates.¹⁸

A study conducted by Rabindra N Misra *et al* over a period of one year from October 2010 to October 2011 highlighted that out of 115 clinically suspected neonatal cases, 75 were found to be culture positive cases. Among these, 75% of proved sepsis cases were preterm and low birth weight neonates. Higher incidence of sepsis in preterm and low birth weight neonates are due to inherent deficiency of both humoral and cellular immunity during the first week of life.¹⁹

Long term complications are more in neonatal sepsis of preterm infants compared to term infant with neonatal sepsis. A study of Rachel E *et al* in Brazil (study period 2012-2013) reported that neurological complication are 2.5 times more in preterm infants with very low birth weight compared to term infants.²⁰

3.2 Causative organism:

The common bacterial isolates for early neonatal sepsis are *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Escherichia coli*. In developing countries, *Staphylococcus aureus* is found to be a significant neonatal pathogen isolated from 8-22% of blood culture.²¹ The etiological bacterial agents differ from developed to

developing countries. In 1990, Group B *Streptococci* was commonest organism for EONS in developed countries. *Escherichia coli* was second common organism.²² Following Group B *Streptococci* prophylaxis, developed countries identified gram negative enteric pathogens as the most common causative organism.²³

A study done by Dar es Salaam *et al* at Muhimbili National Hospital in Tanzania found *Staphylococcus aureus* and *Klebsiella* species to be the most common causes of neonatal sepsis.²⁴

A study carried out in China by Xin-Chuan Chen *et al* found gram positive organism (77.4%) was predominant in neonatal sepsis in Sichuan University.²⁵

Neelam Kaistha *et al* conducted study at Government Medical College Hospital at Chandigarh (study period July 2003 to October 2007) which revealed that gram negative organism were responsible for neonatal sepsis according to 80.40% among 296 positive blood culture.²⁶

According to a study conducted by Rajlakshmi Viswanathan *et al*, in Institute of Post graduate education and Research and Seth Sukhlal Karnani Memorial Hospital Kalkata reported gram negative organism (58%) mainly enteric gram negative bacteria as causative organism among 216 neonatal blood culture samples.²⁷

A study done by Neema Kayange *et al* found that among 149 culture positive cases, 61.1% of sepsis were due to gram negative bacteria. Common bacterial isolates were *Klebsiella pneumoniae*, *Escherichia coli* and *Staphylococcus aureus*. More than 50% of *Klebsiella pneumoniae*, *Escherichia coli* and other gram negative organism were resistant to third generation cephalosporins with majority of them Extended Spectrum Beta

Lactamase (ESBL) producer. Among 32 *Staphylococcus aureus* , 28% of isolates were Methicillin Resistant *Staphylococcus aureus*.¹⁷

A study done by Bambala Puthattayil Zakariya *et al* in Jawaharlal Nehru Institute of Post graduate, Medical Education and Research Puducherry, found 41.6% of blood culture positivity. *Klebsiella pneumoniae* were commonest organism in EONS. *Klebsiella pneumoniae* and coagulase negative *Staphylococcus aureus* were mainly responsible for LONS. 32% of *Klebsiella pneumoniae* isolates were found to be ESBL producers. But all gram negative isolates were sensitive to meropenem.²⁸

A study of Iregu KC *et al* found that 87% of *Klebsiella pneumoniae* isolates were found to be ESBL producers.²⁹

Based on study conducted in North India by Kaistha N *et al* found that 88% gram negative isolates were resistant to gram negative cephalosporin.³⁰

Based on the study conducted by Emily J Welson *et al* at Centres for Disease Control and Prevention, Atlanta and various centre in USA, reported that most common organisms were group B Streptococci (37.8%), *Escherichia coli* (24%), viridans Streptococci (18%), *Haemophilus influenzae*(4%) and *Staphylococcus aureus* (4%). This study showed that group B *Streptococci* as the commonest pathogen of early onset neonatal sepsis.³¹

3.3 Pathogenesis of neonatal sepsis

The normal amniotic membrane, placenta and amniotic fluid by itself serves as a natural protective barrier against infection for inutero fetus by exhibiting anti-microbial effects.³² Fetal systemic bacterial infection may occur in preterm and also in term infants

with the neonates presenting symptomatic at birth itself. This infers bacterial infection may occur at inutero stage. Bacterial infection like *Listeria monocytogenes* is an example for transplacental route of infection affecting neonates via maternal circulation.³³

3.3.1 Pathogenesis of intrauterine infection

Clinical or subclinical maternal infection with agent like cytomegalovirus, Treponema pallidum, Toxoplasma gondii, rubella virus, varicella virus and parvovirus B19 are transmitted to fetus by haematogenous, transplacental transmission. Transplacental infection may occur at any time during pregnancy. Signs of transplacental infection may be noticed immediately after delivery or at later periods variable from months to years. Transplacental infection may result in early spontaneous abortion, congenital anomalies, intra uterine growth retardation, premature birth, stillbirth or asymptomatic persistent infection with sequelae later in life.

First trimester infection may affect organogenesis causing congenital anomalies. Example is congenital rubella. Last trimester infection frequently results in intra partum acquirement of pathogens. In third trimester infection, clinical manifestation occurs sometime after birth.

3.3.2 Pathogenesis of ascending bacterial infection:

Intact amniotic membrane serves as natural protective barrier against the inutero stage infection of fetus. Some anaerobic and aerobic microbes are found as normal

inhabitants of birth passage and those microbes may at times cause ascending bacterial infection.

Chorioamnionitis is microbial infection of amniotic fluid and chorioamniotic membrane. Maternal fever, lower abdominal pain, foul smelling vaginal discharge/amniotic fluid, maternal leukocytosis, maternal and/or fetal tachycardia are main signs and symptoms of chorioamnionitis. Prolonged rupture of membrane (more than 24 hour) may cause chorioamnionitis and in turn ascending infection. Bacterial colonization only does not cause neonatal infection. Prematurity, underlying illness, inoculum size, virulence of infecting organism, the innate immune system and transplacental antibodies are main factors for early neonatal infection. Aspiration or ingestion of bacteria in amniotic fluid, endotracheal intubation, insertion of an umbilical vessels catheter are other factors result in early neonatal sepsis.

A study conducted by Pyati *et al* detected Group B *Streptococci* sepsis among 3000 newborn infants. Nearly all with early neonatal sepsis and a birth weight less than 2000 gram presented with symptoms less than one hour after birth, whereas more than two-thirds of those with a higher birth weight developed symptoms later than four hours.³⁴ These findings indicate that preterm neonates may be exposed to GBS in utero, whereas term neonates often may be exposed during the passage through the birth canal and by aspiration of contaminated amniotic fluid, or by bacteria penetrating through injured skin or natural body openings. In most cases this colonisation proceeds without causing disease. The mechanism by which bacterial colonisation converts to invasive

disease is not fully understood, but it is mainly depends upon bacterial virulence, maternal immunological factors, and the competence of the neonatal immune system.^{35,36}

In the study of Seo *et al*, on EONS (culture proven or clinical) found clinical chorioamnionitis in 34.7% of preterms with a gestational age less than 28 weeks, among 22.2% with gestational age 31-33 weeks, and 9.1% of term neonates. Compared to non-infected deliveries, clinical chorioamnionitis increased the risk of early-onset neonatal sepsis 8 to 10 times.³⁷

3.3.3 Pathogenesis of late onset post natal infection:

In late-onset neonatal sepsis, cases are less likely to have a history of obstetric complications, and may be infected mainly with nosocomial acquired organisms. Post natal infection occurred by direct contact with hospital personnel, the mother, and other family members or from inanimate sources such as contaminated equipment.³⁶

3.4 Immunity:

In both term and pre term infants have decreased function of neutrophils, and decreased complement level. These factors are mainly responsible for neonatal infection. Preterm babies have low immunoglobulin concentration compare to term babies. Maternal IgG antibody is actively transported to fetus through the placenta. These IgG levels are directly proportional to gestational age. Ig M and IgA are not transferred across the placenta, although a fetus can synthesize IgM and IgA in response to intrauterine infection. Neonatal tetanus and Group B Streptococci infections are prevented by

maternal Ig G antibody. IgM antibodies are mainly against gram negative enteric pathogens. Usually newborn infants lack antibody mediated protection against *Escherichia coli* and other Enterobacteriaceae.

3.5 Complement:

The complement system facilitates bactericidal activity against certain organism such as *Escherichia coli* and functions as an opsonin with antibody in the phagocytosis of bacteria such as Group B streptococci. Maternal complement does not cross placenta. A fetus begins to synthesis complement components in first trimester itself. Usually term newborn infants have slightly diminished classical pathway complement activity and moderately diminished alternative pathway activity. In comparison to term infant, preterm infants have low levels of complement components and less complement activities. These deficiencies contribute to diminished complement derived chemotactic activity and to a diminished ability to opsonize certain organisms in the absence of antibody. In neonatal infant, opsonization of *Staphylococcus aureus* is normal, but various degrees of impairment have been noted against *Escherichia coli* and Group B streptococci.

3.6 Neutrophils:

Qualitative and quantitative deficiencies of the phagocyte system mainly contribute to neonatal sepsis.

Causes for increased susceptibility of neonates to infection are stated as

1. Abnormal Neutrophil migration at birth in both term and preterm infants. Specifically telling decreased adhesion, aggregation and deformability of neutrophils may delay prompt response to infection.
2. Abnormal expression of cell membrane adhesion molecules β_2 integrins and selectins and abnormalities in neonatal neutrophil cytoskeleton leads to abnormal chemotaxis and hence impair response to infection.
3. Impaired oxidative respiratory burst of neonatal neutrophils aids in increased risk of sepsis.
4. Decreased storage pool of neutrophil - Frequently noticed neutropenia in preterm and intra uterine growth retarded infants attributes to the increased risk for sepsis.

3.7 Monocyte – Macrophage system:

Prime functions of activated macrophages include antigen presentation, phagocytosis and immune modulation. Although count of monocyte in neonatal blood is normal, function of macrophages such as impaired chemotaxis increases risk of sepsis.

3.8 Natural killer cells:

Natural killer cells (lymphocyte sub group) are cytolytic against cells infected with pathogens. These cells also lyse antibody coated cells and this action is called antibody depended cell mediated cytotoxicity (ADCC). Natural killer cells appear early in gestation and are of equalent numbers as in adults. However diminished cytotoxic activity and ADCC predispose to increased susceptibility of neonates to sepsis.

3.9 Cytokines and acute phase proteins:

Cytokines are endogenous chemical mediators that carry information between different cells and are important factors in the human inflammatory response. They are regulated by a complicated web of regulatory mechanisms including several different cell types. In case of infection, both pro-inflammatory and anti-inflammatory cytokines are upregulated according to a specific time schedule, and so by studying this upregulation in blood samples we can conclude whether systemic inflammation is present or not. Tumor necrosis factor- α (TNF α), Interleukin-1 (IL-1), IL-4, IL-6, IL-8, IL-10, IL-12 and platelet-activating factor are important chemical mediators are released in various inflammatory reactions. Potential marker for bacterial neonatal sepsis, pneumonia and necrotizing enterocolitis are TNF α , IL-6 and IL-8.

Innate immunity also plays an important role against an infectious agent which is due to nonspecific cellular and humoral response. Recognition of pathogens is initiated by soluble components in plasma (including mannose binding lectin) and by recognition of receptors monocyte and other cells.

Cytokines:

Many critical interactions among cells of the immune systems are controlled by soluble mediators called cytokines. The cytokines are a diverse group of intracellular signaling proteins that regulates not only local and systemic immune and inflammatory responses but also wound healing, haematopoiesis, and many other biological processes. Till date, more than 100 genetically unrelated and structurally dissimilar cytokines have been identified. Most of cytokines are glycoproteins and peptides with molecular weights

of between 6000 to 60000. They act at very minimal concentrations of 10^{-10} to 10^{-15} M. So cytokines are highly potent hormone like substances.

Biologically active substances secreted by activated T lymphocytes were called lymphokines. Monocytes and macrophages produce certain types of cytokines which are called as monokines. Some cytokines are normally present in detectable amount in blood and are able to control distant target cells. Examples are transforming growth factor β , stem cell factor, erythropoietin and monocyte colony stimulating factor. Most of other cytokines act either a paracrine manner (act locally near producing cells) or an autocrine manner (directly act on producing cells themselves).

Each and every cytokine is secreted by particular cell types. In response to specific stimuli, these cell types produce cytokines which causes growth, mobility, differentiation or functions of its target cells. There is considerable overlap in the effects produced by different cytokines. Cloning of cytokines and availability of monoclonal antibodies against them has helped to characterize them better.

3.9.1 Interleukin-1 (IL-1):

In 1972, this cytokine was earlier described as leucocyte activating factor, then in 1974 this cytokine described as B cell activating factor and this cytokine was renamed later as interleukin-1 in 1979. IL-1 is a stable polypeptide retaining its activity up to 56°C and between pH 3 and pH 11. IL-1 occurs in two molecular forms. They are IL-1 α and IL-1 β . It is principally secreted by macrophages and monocytes but can be produced by most other nucleated cells as well. Antigens, inflammatory processes, toxins and injury

are stimulating its synthesis. Corticosteroids, cycloserin A and prostaglandins are inhibiting its synthesis.

Stimulation of T-helper cells for production of IL-2 and other lymphokine, B cell proliferation and antibody synthesis, neutrophil chemotaxis and phagocytosis are main immunological effects of IL-1. IL-1 acting on epithelial cells, synovial cells, bone marrow, osteoclasts and vascular endothelium which mediating wide range of physiological, metabolic, haematological and inflammatory changes. It is one of the important endogenous pyrogen.

IL-1 and Tumor Necrosis Factor (TNF) are structurally entirely different cytokines that binds to different unrelated receptor, but their spectrum of biological effects so resemble one another. IL-1 together with TNF is responsible for many of the haematological changes in septic shock and also increases the initial meningeal inflammation in bacterial meningitis. Cytokine inhibitors such as corticosteroids have been found to protect against the sequelae of such excessive meningeal inflammation. IL-1 has beneficial effect in severe infections in immunocompromised hosts.

3.9.2 TNF:

Tumour necrosis occurs in alpha and beta types. This cytokine induces hemorrhagic necrosis in certain tumours and was named as the tumour necrosis factor. The same substance was independently described as cachectin, a serum factor causing the wasting syndrome cachexia during chronic infections. Cachectin renamed as TNF α . Activated macrophages and monocytes are sources of this cytokine. Participation in manifestation of endotoxic shock is main immunological activity of this cytokine.

3.9.3 IL-1 and TNF:

IL-1 and TNF enhance the activation of helper T lymphocytes by antigen presenting cells (APC). APCs, on contact with an antigen, secrete IL-1 and TNF. Both these cytokines provide co-stimulatory signal that promotes Tcell activation. This appear to occur primarily through autocrine effects on the APC itself. By inducing expression of various adhesion molecules, Interferon γ receptors, and class II major histocompatibility complex (MHC) proteins of the surface of an APC, IL-6 and TNF increase the efficiency with which it can bind and activate T cells. Both IL-6 and TNF can promote nearly all types of cellular and humoral responses.

3.9.4 Interleukin-6:

Activated T cells and B cells, macrophages and fibroblasts produce the IL-6. Main functions of IL-6 is synergizing with IL-1 and TNF to co-stimulating immune response by inducing the acute phase protein in liver cells, stimulating the B cell replication, immunoglobulin production and differentiation. Hematopoiesis and thrombopoiesis also stimulated by IL-6. IL-6 has antiviral activity and also cross react with some antisera to interferon β (IFN β). So it called as interferon β 2.

The gene for IL-6 is situated on 7 th human chromosome. Molecular weight of IL-6 ranges from 22000 to 30000.

- IL-6 has a stimulatory effect on liver cells, nerve cells and hematopoietic cells.

Therefore IL-6 is main inducer of the hepatic acute phase response mediator in host defence against infections.

- IL-6 is very effective in increasing TNF or IL-1 induced cachexia and glucocorticoid synthesis.
- IL-6 also stimulates growth of keratinocyte and osteoclastic activities.

A study conducted by Ng PC *et al* revealed that IL-6 had 89% of highest sensitivity and 91% negative predictive (91%) for detecting late onset infection among 101 clinically suspected sepsis cases.⁸⁷

Another study from Istanbul University, Turkey done by Emine Kocabas *et al* showed that IL-6 had 96.2% of sensitivity, 89.7% specificity, 86.2% of positive predictive value and 96.1% of negative predictive value.⁸⁴

A recent study (2013) from Iran, done by Hassan Boskabadi *et al* found that IL-6 had 92.5% of sensitivity, 96.6% of specificity, 97% of positive predictive value and 93% of negative predictive value.⁸⁶

3.9.5 Interleukin-2:

Interleukin-2 is produced by activated T lymphocytes. This cytokine is essential for T-lymphocytes proliferation. IL-2 was first described in 1976 by its ability to enhance mitogenesis of human T cells and to support continuous growth of normal T cells in culture. So this cytokine is called T cell growth factor. It was an important discovery in immunology and it made it possible to propagate and study individual clones of normal T lymphocytes that maintain their immunological properties. It converts large granular lymphocytes into lymphokine activated killer cells, which can destroy NK-

resistant tumour cells. This property can be used in the treatment of certain types of cancers. IL-2 also stimulates secretion of other lymphokines.

3.9.6 Interleukin-3:

Interleukin-3 is a growth factor for bone marrow stem cells produced by T-lymphocytes. It stimulates multilineage haematopoiesis, and therefore it is also known as the multicolony stimulating factor.

3.9.7 Interleukin-4:

Interleukin-4 is produced by T-helper cells. It acts as B-cell differentiating factor, also acts as a growth factor for T cells and mast cells and enhances the activity of cytotoxic T cells. It increases synthesis of IgG-1 and IgE and play a role in atopic hypersensitivity.

3.9.8 Interleukin-5:

Interleukin-5 is produced by T-helper cells. It causes the proliferation of activated B cells and eosinophills and stimulates production of IgA and IgM.

3.9.9 Interleukin-7:

Interleukin-7 is produced by the spleen, bone marrow stromal cells. It acts as B cells and T cells growth factor.

3.9.10. Interleukin-8:

Interleukin-8 is produced by macrophages and other cells. It acts as neutrophil chemotactic factor.

3.9.11. Interleukin-9:

Interleukin-9 is produced by T-helper cells. It helps in T cells and B cells growth and proliferation.

3.9.12. Interleukin-10:

Interleukin-10 is produced by T and B cells and macrophages. It inhibits interferon production and functions of mononuclear cells.

3.9.13. Interleukin-11:

Interleukin-11 is produced by bone marrow stromal cells. It induces acute phase protein.

3.9.14. Interleukin-12:

Interleukin-12 is produced by T cells. It activates Natural Killer (NK) cells.

3.9.15. Interleukin-13:

Interleukin-13 is produced by T cells. It inhibits functions of mononuclear cells.³⁸

Clinical features of bacterial neonatal sepsis:

3.10. Clinical features of neonatal sepsis:

Clinical features of neonatal sepsis are mainly variable. Clinical features of neonatal sepsis is divided into non-specific features and specific features.

Non-specific features: The earliest signs of sepsis are frequently subtle and nonspecific.

Clinical diagnosis needs a high index of suspicion for early diagnosis.

Clinical features are “hypothermia or fever, lethargy, poor cry, refusal to suck, poor perfusion, prolonged capillary refill time, hypotonia, absent neonatal reflexes, brady/tachycardia, respiratory distress, apnea and gasping respiration, hypo/hyperglycemia and metabolic acidosis”.

Early manifestation of neonatal sepsis may involve only one system and present with limited symptomatology. Initial signs and symptoms of neonatal sepsis are

temperature instability (hypothermia or fever), refusal of feeding and edema. Signs and symptoms related to respiratory system are apnea, tachypnea, grunting, cyanosis, retractions of chest wall and nasal flaring. Main signs and symptoms related to cardiovascular system are pallor, cold and clammy skin, tachycardia (more than 160 beats /min) or bradycardia (less than 100 beats /min) and hypotension. Signs and symptoms related to central nervous systems are lethargy, irritability tremors, convulsion, abnormal Moro reflex and hypotonia. Abdominal distension, vomiting, diarrhea and hepatomegaly are main signs and symptoms related to gastro intestinal tract. Oliguria is main symptom related to renal system. Signs and symptoms related to haematologic systems are jaundice, splenomegaly, pallor, petechial purpura and bleeding. Signs and symptoms related to skin and soft tissue are impetigo, omphalitis, scalp abscess, fasciitis, adenitis and abscess of cystic hygroma. Most of the times, various non-infectious diseases can co-exist with neonatal sepsis, which in turn makes sepsis diagnosis a tough one. Surfactant deficiency leading to respiratory distress syndrome can coexist with bacterial pneumonia.³⁹

3.11. Clinical criteria for neonatal sepsis:

3.11.1. Integrated Management of Childhood Illness criteria:

“Tachypnea (> 60 breath per minute), nasal flaring, increased chest retraction, grunting, nasal flaring, bulging fontanel, pus draining from ear, redness around umbilicus, temperature instability (>37.7°C or <35.5°C), lethargic, reduced movements, not able to feed and convulsions” are components of integrated management of childhood illness criteria for neonatal sepsis.

3.11.2. WHO criteria:

“Convulsion, tachypnea (> 60 breath per minute), severe chest retraction, temperature instability ($>37.7^{\circ}\text{C}$ or $<35.5^{\circ}\text{C}$), lethargic, reduced movements, not able to feed, crepitation and cyanosis” are main components of WHO criteria for neonatal sepsis.⁴⁰

A study conducted by Jaswal RS *et al*, in Shimla medical collages India, found that the most frequent clinical presentation of neonatal sepsis were respiratory distress, lethargy and jaundice with combined frequency of 40% followed by fever and poor feeding.⁴¹

Tanzania based study by Arif SH *et al*, stated the most common clinical presentation found in neonatal sepsis were fever reported in 91% of neonates, inability to breast feed, bulging anterior fontanelle, dyspnea, jaundice, and seizures. Few other clinical features of neonatal sepsis included abdominal distension, tachycardia, tachypnea, disseminated intravascular coagulopathy and abscesses.⁴²

3.12. Diagnosis of neonatal sepsis:

Neonatal sepsis is a potentially dangerous and serious condition. That can cause increased mortality and morbidity very rapidly, if not treated correctly and quickly. The ideal test to confirm neonatal sepsis should have 100% sensitivity and 100% specificity. But such a test is unlikely to be discovered till date, due to non-specific signs of neonatal sepsis. Many signs and symptoms of neonatal sepsis are also present in some of non-infectious conditions like acute respiratory distress, aspiration of amniotic fluid and hypoglycemia etc.⁴³ Hence it remains a challenge for physicians to correctly diagnose

neonatal sepsis in a timely manner. So a rapid reliable diagnostic test for neonatal sepsis is essential in order to initiate treatment in suspected neonates on time so as to reduce associated morbidity and mortality.

In routine clinical practice, the recommended approach is to liberally start intravenous antibiotics and then perform a ruling out procedure that normally lasts for several days. If all the tests for neonatal sepsis are negative and neonate has recovered fully, the antimicrobials can be discontinued and infant can be discharged from the neonatal intensive care unit. This rule out procedure normally includes various cultures such as blood, cerebrospinal fluid, urine etc, x-rays and various markers of sepsis. If it were possible to decrease the time taken by this investigation, the benefits would be obvious in terms of reduced costs of treatment, reduced infants suffering and reduced duration of antibiotics. So, there is a great need for specific and less time consuming diagnostic methods.

A study conducted by Ng PC *et al* in 2004, presented a list of 58 different laboratory tests that had already been evaluated as diagnostic tests for neonatal sepsis.⁴⁴

Another study of review article by Pierrakos *et al* reviewed 3370 references covering 178 biomarkers.⁴⁵

3.12.1. Blood cultures:

Gold standard diagnostic test for suspected neonatal sepsis is blood culture. A small blood volume is enough for isolation of bacteriological agents as low as 0.2 to 0.5mls. But, increased blood volume 1 to 2mls is required for detection of low bacteraemia particularly where there is history of prior use of antibiotics. Venous blood is

routinely used for blood culture. The skin should be prepared with disinfectant solution before venipuncture. But care must be taken; disinfectant solution does not harm skin of newborn infants.⁴⁶

A study conducted in 1997 by Kellogg *et al* revealed that low level bacteremia was very common in infants. So they recommended a sample volume of 6 ml for to get optimize sensitivity. Though, this would represent around 4.5% of an infant's blood volume.⁴⁷

Volume of blood needed for culture depends up methods. Automated blood culture systems such as BacT/Alert required small volume of blood such as 0.5ml only. But 1-2 ml needed for conventional method.

Schelonka RL *et al* found that, if one or two viable colony-forming units are in the blood inoculated into culture media, the BacT/Alert system will detect growth rapidly. Since there appears to be a sizable subset of neonates who are at risk of sepsis with a colony count less than 4 CFU/ml, then a 0.5 ml inoculum of blood into the culture media is insufficient for sensitive and timely detection of bacteremia. One to two milliliters of blood should increase microorganism retrieval in the face of low-colony-count sepsis by conventional blood culture method.⁴⁸

In developing countries, the conventional type of blood culture method is commonly used. Because it is less expensive to do when compared to automated culture systems. But the procedure is labour intensive and the yield is significantly low sensitivity than that of an automated system.⁴⁹ In developed countries, automated systems

are mostly used. Main advantages of these techniques are the blood culture to be monitored continuously and resulting in a shorter time to identify a positive culture.⁵⁰

A study conducted by Baltimore RS *et al* in Yale University of Medicine, New Haven, USA “found ninety-four cases of non-GBS early-onset sepsis were detected between 1996 and 1999. The rate of GBS-related early-onset infection reduced from 0.61/1000 to 0.23/1000 births, but the annual rate of non-GBS sepsis remained steady, ranging from 0.65 to 0.68/1000 during the surveillance period. There was an increase in the proportion of *Escherichia coli* infections that were ampicillin resistant between 1996 and 1998, but the proportion decreased in 1999”.⁴⁰

A study (during 1998 to 2004) conducted by Ramesh Bhat *et al* highlighted that out of 2182 samples received from clinically suspected cases of early onset neonatal sepsis 389 (17.8%) showed positive blood culture.²²

A study by Subhranshu Sekar Kar *et al* (2007–2010) revealed that, among 160 blood culture samples tested, 60(16.2%) were blood culture positive. This study was done at Hi-Tech medical college, Bhubaneswar, Odisha.⁵¹

A study conducted in Kolkatta during March 2009 –August 2010 by Rajalakshmi Vishwanathan *et al* show 46.3% of positive blood culture in 216 samples.²⁷

A study done by Sucila Thangam *et al* in Tamiladu during April – September 2010 revealed 28% of positive blood culture in 50 samples.¹⁶

Based on the study conducted by Shrestha R K *et al* in Nepal medical college, Kathmandu, out of 120 suspected cases, 37 (30.8%) were found to be blood culture positive during the period of July 2011 to January 2012.⁵²

A study conducted by Neelam Kaistha *et al* at Government Medical College Hospital, Chandigarh found that 296 (13.17%) blood culture positive among 2247 of blood sample from clinically suspected neonatal sepsis.²⁶

Even though, if ideal blood volumes are used, blood culture has obvious limitations in sensitivity. A negative blood culture report alone cannot support withdrawal of antibiotic treatment if the neonate's clinical condition indicates ongoing sepsis. So blood cultures have limited sensitivity and this method is time consuming, and most microbiology laboratories will take one week for complete report.⁵³

3.12.2. Haematological marker scoring system:

This test can be used for screening for neonatal sepsis. It can be performed easily and it is readily available in most of the settings. It is usually a combination of various parameters from complete blood picture. Various parameters are

- “Total white blood cell count
- Absolute Neutrophil count (ANC)
- Immature Neutrophil : Total Neutrophil Ratio
- Platelet count
- Micro erythrocyte sedimentation rate
- C-reactive protein”.⁵³

In complete blood count, total leukocyte count, neutrophils and platelets are predictors of ongoing infection. Ongoing infection interpreted by extreme value of these parameters.^{53,54}

Non-infectious causes such as asphyxia, maternal fever and post gestational age are elevating these parameters. This factors causes difficult in interpretation of results.⁵⁵

Total white cell count alone could not be considered valid for confirmation of neonatal sepsis. The reasons being are its grossly varied values and narrow transition between normal and abnormal values. Neutropenia is seen more in sepsis rather than neutrophilia. This neutropenia results from significantly raised adherence of neutrophils to surface of endothelial cells and its increased consumption at the area of infection. Neutropenia also being noted in certain other condition such as inborn errors of metabolism and asphyxia neonatorum makes it of limited value as a sole marker of sepsis. Frequently neonatal sepsis is associated with low absolute neutrophils count, and high I/T ratio. Low white blood cell count are more helpful if obtained after 4 hours of life due to normal increase of white blood cell and neutrophil count after 6 hours of life.^{56,57}

Hematological marker scoring interpretation:

- “Total leucocyte count: less than 5000/mm³ or more than 30000/mm³
- Absolute Neutrophil Count (ANC): less than 1000/mm³
- Immature / Total neutrophil ratio: more than 0.2
- Micro Erythrocyte sedimentation rate: more than 15 mm in hour.
- C-reactive protein: more than 1mg/dl”.

If two or more abnormal hematological markers are present, it should be considered as a positive screen for neonatal sepsis. If the hematological marker score is negative, but clinical suspicion persists, it should be repeated within 12 hours. If the hematological markers are still negative, neonatal sepsis can be excluded. Presence of two abnormal hematological markers are associated with 93-100% sensitivity, 83% specificity, 27% positive predictive value and 100% negative predictive value in neonatal sepsis.⁵⁶

Neonatal sepsis also causes thrombocytopenia, because of disseminated intravascular coagulation and the damaging effects of endotoxin on platelets. Therefore combining the parameter of the complete blood count is the form of a Hematological markers Scoring System that has been suggested and can serve as a screening tools.⁵⁸

3.12.3. C-Reactive Protein:

Tillet and Franchis of Rockefeller University were firstly described C - reactive protein. They demonstrated a precipitation reaction with polysaccharide fraction C from the pneumococcal cell wall and serum of patient suffering from pneumococcal pneumonia. Serum of healthy controls and some pneumococcal pneumonia recovered patients does not show this precipitation. In interpretation of the fact that the polysaccharide fraction was a protein, the C-reactive component in the serum was named C-reactive protein.⁵⁹ In 1950, CRP had been detected in more than 70 various disorders including acute bacterial, viral, and other infections, as well as noninfectious diseases such as, rheumatic disorders, acute myocardial infarction, and various malignancies. All of these disorders of different

etiology had in common the factor of inflammation and/or tissue injury. Increased serum level of CRP is very early and sensitive response to most of microbial infections.⁶⁰

C- reactive protein (CRP) is one of the acute-phase proteins. It belongs to the pentraxin family of ligand-binding and calcium-dependent plasma proteins. In acute infection, serum level of CRP increased up to 50 to 100 mg/L. But in chronic condition like rheumatoid arthritis and atherosclerosis its level generally remains below 10 mg/L.⁶¹

Synthesis and metabolism:

Hepatocytes are main site for production of CRP. CRP synthesis and secreted depends upon various response to cytokines such as Interleukin-6, Interleukin-1 and Tumour Necrosis Factor- α (TNF- α). CRP is primarily derived via IL 6- dependent hepatic biosynthesis. Increased CRP level in neonate always represents endogenous synthesis. CRP passes through the placenta is very very minimal. Only single stimuli enough to hepatic synthesis of CRP, that increase the serum concentration above 6mg/l by about 6 hours and peaking at around 48 hours.⁶²

The stimulatory effects of cytokines on the production of acute phase proteins increased by glucocorticoids.⁶³ Insulin, on the other hand, decreases their effects on the production of some acute phase proteins.^{64,65}

CRP detection method:

A large number of methods are available for the detection of CRP and estimation of CRP level in the serum. Even though, nephrometry, electroimmunoprecipitation assay

and immunometric assay are sensitive and quantitative method for the estimation of CRP. These methods have complicated procedure, so these tests done only well-equipped laboratories only.⁶⁰ Latex agglutination test is the alternative test, it have a quiet sensitive, rapid method to detect serum CRP in qualitatively and semi quantitatively. The serum CRP concentration of 6mg/L or more was considered as positive.(15)

Function of C-reactive protein:

The main receptor to CRP is phosphocholine. Phosphocholine is found in lipopolysaccharide of bacterial cell wall and in most of biological membrane. CRP and phosphocholine binds first, after that CRP is recognized by the complement system. CRP activates the complements system and promotes the phagocytosis by neutrophils and macrophages. Then CRP initiates release of proinflammatory cytokines.^{70,71}

The sensitivities and specificities of CRP assay in the detection of neonatal sepsis using culture as a gold standard. Sensitivity of CRP is more important than specificity in detection of EONS and LONS.

Condition where CRP is elevated:

CRP level is increased in some acute conditions such as bacterial infection, bacterial endocarditis, pneumococcal pneumonia and acute rheumatic fever. CRP level is increased in more number of chronic condition like polyarthritis nodosa, rheumatoid arthritis, systemic lupus erythematosus, Inflammatory bowel disease, acute myocardial ischemia and malignancies etc.⁶⁵

A study conducted by Benitz MD *et al* from Stanford University of Medicine shows 54.6% of sensitivity on proven neonatal sepsis and 65.5% of sensitivity in probable neonatal sepsis among 1002 infants. The positive predictive value was 99.7% and negative predictive s for CRP was 98.7% for conformed neonatal sepsis.⁶⁶

Laborada G *et al* revealed that during study (2003), out of 105 neonatal sepsis cases blood culture tested 48 cases were positive by automated technique. This study also shows 69% sensitivity , 96% specificity, 93% positive predictive value and 80% negative predictive value.⁶⁷ A similar study by Doellner H *et al* reported that CRP sensitivity 63%, specificity 97%, positive predictive 83% and negative predictive value 91%. Doellner H et al study also include 36 samples are positive among 253 blood culture.⁶⁸

According to a study conducted by Franz A R *et al* revealed that 46 cases are culture positive among 162 neonates with suspected sepsis and also reported 28% of CRP sensitivity, 97% of specificity, 81% of positive predictive and 77% of negative predictive value.⁶⁹

Neonates with septicemia due to gram negative organism have higher serum CRP level than gram positive organism.⁷²

Recent study by Flora Chacha *et al* highlighted that out of 305 samples received from clinically suspected cases of neonatal over a period of 2 years, 104 cases showed CRP positive. This study also revealed CRP sensitivity 90% , among these 75%

higher sensitivity for gram negative septicemia compare to 50% sensitivity for gram positive septicemia.¹⁵

A study conducted in Thailand by Nuntnarumit P *et al* found that serial quantitative CRP measurement were found to have better predictive than complete blood count with 100% sensitivity, 94% specificity, 91% positive predictive and 100% negative predictive.⁷³

A study was done in Rawalpindi Pakistan by Khassawneh M *et al*, comparing CRP, absolute neutrophil count and I/T ratio, CRP was found to have a specificity of 95% in diagnosing neonatal sepsis followed by absolute neutrophil count.⁷⁴

A study conducted by Kohli-Kochhar R *et al*, in Port Harcourt Nigeria, the sensitivity, specificity, positive predictive value and negative predictive of serial CRP measurements were found to be 74.0%, 74.1%, 68.0% and 79.0% respectively in the diagnosis of neonatal sepsis using blood culture as the gold standard.⁷⁵

In a study done by Hofer N *et al*, comparing CRP, interleukin 6 and immunoglobulin M; revealed that CRP was the best among the three with 95% sensitivity and 98% NPV in the diagnosis of early gram negative sepsis.⁷⁶

Serial serum CRP measurements taken between 24 and 48 hours after the onset of infection have been found to have high sensitivity for probable septicemia. Hence serial CRP is suggested for the diagnosis of neonatal sepsis to predict early infection.⁷⁷ The neonates who were admitted with clinical features of neonatal sepsis and started on

empirical treatment with antibiotics following the negative results of CRP, the physician can stop the antibiotics thus can minimize antibiotic exposure and shorten hospital stays. The diagnostic value of serial measurements of serum CRP levels can also be used for monitoring the severity of sepsis and improvement after initiation of treatment.⁷⁸

3.12.4. Procalcitonin:

Procalcitonin is another acute phase protein which is made up of 116 amino acids. It is a precursor of calcitonin. Within 6-8 hours of bacterial infection, bacterial endotoxin stimulates monocytes and hepatocytes which produce procalcitonin. Its level reaches peak at 6 – 8 hours, and stays minimum for a day. Its half-life is up to 30 hours. Procalcitonin level is increased during infection in neonates, children and adults. Serum procalcitonin level is more increased in bacterial infection than in viral infection. In early neonatal bacterial infection, procalcitonin is more sensitive than CRP.⁷⁹ Serum procalcitonin level also increased in some non-infectious conditions such as neonates with respiratory distress syndrome, haemodynamic instability and diabetic mothers.⁷⁹

A study done by Cetinkaya M *et al*, during the period of 2008-2009 found that the serum procalcitonin levels were higher in the neonatal septic cases compared with the non-septic cases. This also revealed procalcitonin and CRP had sensitivity of 97%, specificity of 91%, positive predictive value 96% and negative predictive value of 87%. The inference was procalcitonin more than 2.3 ng/ml or CRP more than 30 mg/l denotes a possibility of EONS and LONS. In such conditions antimicrobial treatment need to be carried over in the absence of positive culture.⁸⁰

3.13. 1.Prevention strategies for EONS:

Prior administration of parental antibiotics to antenatal mothers prevents EONS by group *B Streptococci* to a great extent. Ampicillin or cefazolin are used as prophylactically four hour before delivery. In mother who have mild pencillin allergy, cefazolin may be administrated. In case of serious pencillin allergy, clinadamycin is the drug of choice. In clindamycin resistant cases, vancomycin is used as an alternative drug for prophylaxis.⁸¹

Indications for intranatal antibiotics are

- Positive antenatal culture for group B streptococci
- Premature rupture of membranes. (>18 hours)
- Previous infection with group B Streptococci.

3.13.2.Prevention strategies for LONS:

- To provide practical training for hand washing technique for entire health care team.
- To provide adequate soap and running water facilities.
- To prepare standard operating procedure for all invasive methods.
- To carry out regular meeting with infection control committee to monitor infection rate.
- To ensure the adequate of physicians and nurses per bed according to current recommendations.⁸¹

4. MATERIALS AND METHODS

The present study was conducted at the Department of Microbiology , Tirunelveli Medical College , Tirunelveli from Jauary 2015 to August 2015.

4.1 Study group

A total of 100 clinically suspected sepsis cases in neonates (0 day to 28 days)

4.2. Inclusion criteria

- Neonates who were admitted in Neonatal Intensive Care Unit at Tirunelveli Medical College with signs suggestive of sepsis, or those who developed signs of sepsis while they were in the ward.

4.3. Exclusion criteria:

- Neonates who were on antibiotics,
- Neonates who had birth asphyxia and aspiration syndromes,
- Neonates who had congenital anomalies and inborn errors of metabolism.

4.4. Ethical clearance

Ethical clearance was obtained from the college ethical committee before the commencement of the study.

4.5. Consent

Informed consent was obtained from reliable informants of neonates who participated in the study.

4.6. Proforma:

The proforma was filled with the details like name of mother of neonates, age (in hours or days), sex, weight of neonates at time of birth, gestational age (in weeks), mode of delivery and clinical diagnosis and other parameters relevant to the present study.

4.7. METHODS

Blood samples were taken from 100 clinically suspected neonatal sepsis and were tested for blood culture, detection of serum level of CRP by latex agglutination test and detection of serum level of IL-6 by ELISA.

4.7.1. Blood collection method:

Ideal blood sample collection should be done before initiation of anti-microbial agents.

Volume of blood needed for culture

Amount of blood needed for cultures for neonates is significantly lower than that needed for adults because neonates tend to have a higher concentration of bacteria in their bloodstream than adults. Hence 2ml of blood was usually considered as the standard volume of blood adequate to detect bacteremia in neonates.⁴⁸

- Proper aseptic precautions were undertaken during blood specimen collection to avoid sample contamination.

- With clean gloved hands, preliminary aseptic precautionary steps like cleansing the venipuncture site with 70% ethanol and 2% tincture iodine and proper drying were followed.
- Then using a 2ml syringe with a 28G needle about 2-3 ml of blood was aspirated. Immediately and without changing or contaminating the needle 2 ml of blood sample was transferred into the top of the blood culture bottle that contains 20ml brain heart infusion broth. (HiMedia, India)
- Another 1 ml of blood was collected in serum separating vial.
- Sharps were disposed in a sharps container.
- The culture blood was gently mixed and labelled.
- The inoculated bottles were sent to the laboratory immediately.
- The collected samples were subjected to various laboratory studies.

4.7.2. Storage of serum sample:

Blood samples were centrifuged within 30 minutes of collection. Serum samples were immediately tested for CRP by latex agglutination method and then stored for IL-6 ELISA at -80 °C.

4.7.3. Blood culture processing procedure:

Inoculated culture bottle was incubated at 37°C for up to 7 days. Subsequent sub culture was done in solid agar plates such as blood agar plate, chocolate agar plate, Mac conkey agar plate and nutrient agar plate after 24 hours and 72 hours with last subculture

being done after seven days. Subculture blood agar plate, Mac Conkey agar plate and nutrient agar plate were incubated aerobically and chocolate agar plate was incubated in carbon dioxide atmosphere for 24 hours.

4.7.4. Identification of microorganism:

- The isolates were routinely identified by standard bacteriological techniques.
- Morphology of colonies on nutrient agar plate, Mac Conkey agar plate and blood agar plate were studied.
- Mac Conkey agar plate was used to differentiate lactose fermenting from non-lactose fermenting organisms. Lactose fermenting organism such as *Escherichia coli* and *Klebsiella* species which produced pink colour colonies were isolated. Non -lactose fermenting organism such as *Pseudomonas* species which produced colourless colonies were isolated.
- Blood agar plate was used to differentiate α -haemolytic from β -haemolytic organisms. *Staphylococcus aureus* producing β -haemolysis were identified.
- Using nutrient agar plate *Staphylococcus aureus* producing golden yellow colour colonies and *Pseudomonas aeruginosa* producing metallic shin along with bluish-green diffusible pigment were identified.
- Using gram stain, gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli*, *Klebsiella* species and *Pseudomonas aeruginosa*) organism were differentiated. Shape and arrangements of organism whether cocci or bacilli

and in pairs, chains or clusters were studied. Colonies were further tested for catalase, oxidase and motility tests.

- Motile organisms such as *Escherichia coli* and *Pseudomonas aeruginosa* and non-motile organism such as *Klebsiella* species were identified.
- Biochemical tests such as indole test, methyl red test, Voges-Proskauer test, Triple Sugar Iron test, citrate utilization test, various sugar fermentation test and Oxidation-Fermentation test (OF Test) were used to differentiate organisms among Enterobacteriaceae family and *pseudomonas spp.*⁹⁰
- Some specific test like catalase test, coagulase test and phosphatase test for *Staphylococcus aureus* were done.

All blood samples were subjected to the above said various tests and causative organisms for neonatal sepsis were identified.⁹⁰

4.7.5. ANTIBIOTIC SUSCEPTIBILITY TESTING:

The antibiotic susceptibility testing was done in all isolates by Kirby Bauer disc diffusion method according to the CLSI guideline.

Kirby-Bauer's disc diffusion method:

About 3-5 colonies of the test organism were inoculated in 2 ml of peptone water and incubated for 2-4 hours at 37°C. The turbidity of the inoculum was adjusted to 0.5 McFarland standards (1.5×10^8 CFU/ ml). A sterile cotton swab was soaked in the inoculum and a lawn culture was made on to the Muller-Hinton agar (MHA). By rotating the swab against the inner side of the test tube, excess broth was expressed. The panel of

antibiotic discs was applied and incubated at 37°C for 18-24 hours. The zone size was recorded and interpreted as per the CLSI guidelines 2013.

The three interpretive categories are described as follows.

Susceptible:

This indicates that the recommended antibiotic in appropriate dose for recommended period is the appropriate agent for treating the infection.

Intermediate:

This indicates that the tested organism may be inhibited by possible concentrations of certain drugs if higher concentrations of the drug can be used safely.

Resistant:

The antibiotic tested may not be an appropriate choice for the infection against the tested organisms either they are not inhibited by the concentration of the drug normally achievable with the recommended dose or because the test result vastly correlates with a resistance mechanism.

In this study the susceptibility for the organism was tested against following antimicrobials from Hi-media laboratories Ltd, Mumbai.

Interpretation of Antibiotic susceptibility testing

Antibiotic	Concentration (in µg)	Sensitive (in mm)	Intermediate (in mm)	Resistant (in mm)
Ceftriaxone	30	>23	20-22	<19
Cefotaxime	30	>26	23-25	<22
Ceftazidime	30	>18	15-17	<14
Gentamicin	10	>15	13-14	<12
Amikacin	30	>17	15-16	<14
Ciprofloxacin	5	>21	16-20	<15
Ofloxacin	5	>17	14-16	<13
Meropenem	10	>19	16-18	<15
Pip – Tazo	100	>21	15-20	<14
Ampicillin	10	≥18	14-17	≤13
Cefoxitin	30	≥18	15-17	≤14
Vancomycin	30	≥15		

Gentamycin, amikacin, cefotaxim, ceftazidime, ciprofloxacin are first line drugs for gram negative organisms. Imipenem, meropenem and cefaperazone-sulbactum are reserved for second line drugs.

Ceftazidime (30µg) and ceftazidime – clavulanic acid (30µg / 10µg) disks were used for detection of extended spectrum β -lactamase (ESBL) in enterobacteriaceae. The above said both disks were placed 20 mm apart in Mueller-Hinton agar and incubated at 37°C for 24 hours. A zone size ≥ 5 mm in ceftazidime – clavulanic acid combination disk compared to ceftazidime alone disk was interpreted as positive for ESBL production.

Ampicillin, cefoxitin gentamicin, amikacin, ciprofloxacin and vancomycin were tested for gram positive organism. Pencillin and Cefoxitin disc were used for detection of Methicillin Resistant *Staphylococcus aureus* (MRSA).

4.8. Serum CRP level detection by latex agglutination test:

All the 100 samples were tested for CRP detection by latex agglutination test with the help CRP test kit of SPAN DIAGNOSTICS LTD, INDIA

4.8.1. Principle:

Specially selected polystyrene latex particles are coated with monospecific goat anti human CRP antibodies. When a serum positive for C - reactive protein is mixed with the latex reagent, a positive result is indicated by a distinctly visible agglutination of the latex particles in the test cell of the slide used. In specimen negative for C – Reactive Protein, the latex remains in a smooth suspension form in the test cell.

4.8.2. Materials provided:

C - reactive protein latex agglutination test kit contains the following items to perform the assay.

- Latex reagent for tests – Suspension of polystyrene latex particles, coated with monospecific goat anti-human CRP antibodies.
- Positive Control serum – 0.5ml
- Negative Control serum – 0.5 ml

- Disposable slides with 8 test cells.
- Disposable mixing sticks.
- Disposable plastic droppers with a rubber teat.

4.8.3. Storage

C-Reactive Protein latex agglutination kit was stored at 2-8 °C.

4.8.4. Test procedure:

Qualitative analysis:

- The latex reagent, controls and serum specimens were brought to room temperature. The antigen suspension was mixed thoroughly prior to use.
- One drop each of patient serum, positive and negative control sera were placed in respective cells of the test plate.
- Then one drop each of CRP latex reagent was added to each of these sera.
- The sera and latex reagent were mixed with separate mixing sticks and the fluid spread over the entire area of the particular cells.
- The test slide was tilted back and forth for two minutes so that the mixture rotates slowly inside the cells .
- At the end of two minutes the results were read under bright light.

4.8.5. Interpretation of results:

- Strong Positive – Distinct coarse agglutination occurs within 0.5 minute.
- Weakly Positive – Fine agglutination usually taking full 2 minutes.
- Negative - No agglutination.

Distinct agglutination indicates CRP content of more than 6 mg/litre in undiluted serum specimen. Sera with positive results in the screening were tested in the titration test.

4.8.6. Semi quantitative analysis:

0.9% saline solution was prepared. Then the specimen was diluted with saline until the last dilution giving distinct agglutination.

Dilution	CRP (mg/litre non-diluted specimen)
1:2	12
1:4	24
1:8	48
1:16	96
1:32	192

Titre is the last dilution step giving visible agglutination. Read the titre in the last dilution step with agglutination and multiply the titre with the conversion factor 6 to get the results in mg/litre.

With the above standard guidelines, samples were tested and obtained results were documented.

4.9. Serum IL-6 level detection by ELISA:

All the 100 samples were tested for IL-6 detection by ELISA with the help of DIACLONE SAS, FRANCE ELISA test kit.

4.9.1. Principle of the method:

A capture antibody highly specific for Interleukin-6 has been coated to the wells of the microtitre strip plate provided during manufacture. Binding of IL-6 samples and known standards to the capture antibodies and subsequent binding of the biotinylated anti-IL-6 secondary antibody to the analyte is completed during the same incubation period. Any excess unbound analyte and secondary antibody is removed. The HRP conjugate solution is then added to every well, following incubation excess conjugate is removed by careful washing. A chromogen substrate is added to the wells resulting in the progressive development of a blue coloured complex with conjugate. The colour development is then stopped by the addition of acid turning the resultant final product yellow. The intensity of the produced coloured complex is directly proportional to the concentration of IL- 6 present in the samples and standards. The absorbance of the

colour complex is then measured and the generated OD values for each standard are plotted against expected concentration forming a standard curve. This standard curve can then be used to accurately determine the concentration of IL – 6 in any sample.

4.9.2. Materials provided:

- A capture antibody highly specific for IL-6 Coated Microwells(12×8 wells)
- Biotinylated anti-IL-6 – 0.4ml X 1
- Biotinylated Antibody diluent – 1 X 7 ml.
- Streptavidin – HRP : 2 x 5 µl
- HRP Diluent : 1x23 ml
- TMB Substrate.
- Stopping solution:1.6 N sulfuric acid.
- Standard : 200pg/ml. (2 vials)
- Standard Diluent: 1x7ml.
- Control: 2 (Freeze dried powder form).
- Washing Buffer: 200x concentrate dilute in distilled water.

4.9.3. Material required:

- Microtitre plate reader with appropriate filters (450 nm required with optional 620nm reference filter).
- Microplate washer
- 10, 50, 100, 200 and 1000µl adjustable single channel micropipettes with disposable tips.

- 50 -300 µl multi-channel pipette and reagent reservoirs.
- Distilled water.
- Vortex mixer.

4.9.4. Kit storage:

Store kit reagents between 2 and 8°C. Immediately after use remaining reagents should be returned to cold storage (2-8°C).

Preparation of Standard :

Standard vials must be reconstituted with 770 µl of standard diluent on the vial immediately prior to use. This reconstitution gives a stock solution of 200pg/ml of IL-6. Mix the reconstituted standard gently by repeated aspiration. Serial dilutions of the standard are made directly in the assay plate to provide the concentration range from 200 to 6.25pg/ml.

- Immediately after reconstitution add 200µl of the reconstituted standard to well's A1, which provides the highest concentration standard at 200pg/ml.
- Add 100µl of appropriate standard diluent to the remaining standard wells B1 to F1.
- Transfer 100µl from wells A1 to B1. Mix the well contents by repeated aspirations and ejections taking care not to scratch the inner surface of the wells.
- Continue this 1:1 dilution using 100µl from wells B1 through to F1 providing a serial diluted curve ranging from 200pg/ml to 6.25pg/ml.
- Discard 100µl from the final wells (F1) of the standard curve.

4.9.5. Preparation of Controls:

Freeze-dried control vials should be reconstituted with 1ml of standard diluent – Human serum. This is used as Positive control (G1). Only 100µl of standard diluent - Human serum used as Negative control.

Preparation of Biotinylated anti-IL-6:

Biotinylated anti-IL-6 is prepared immediately before use. Dilute the biotinylated anti-IL-6 with the biotinylated antibody dilute in an appropriate clean glass vial using volumes appropriate to the number of required wells.

No. of wells required	Biotinylated Antibody (µl)	Biotinylated Antibody Diluent (µl)
16	40	1060
24	60	1590
32	80	2120
48	120	3180
96	240	6360

4.9.6. Preparation of Streptavidin - HRP

To centrifuge vial of Streptavidin-HRP vial for a few seconds in a microcentrifuge to collect all the volume at the bottom. Then the 5 µl vial with 0.5ml of HRP diluent immediately before use. Further dilute the HRP solution to volumes appropriate for the number of required wells in a clean glass vial.

No. of wells required	Streptavidin – HRP (μ l)	Streptavidin – HRP Diluent (ml)
16	30	2
24	45	3
32	60	4
48	75	5
96	150	10

4.9.7. Preparation of wash buffer:

Dilute the (200x) wash buffer concentrate 200 fold with distilled water to give a 1x working solution. Pour entire contents of the washing buffer concentrate into 2000 ml graduated cylinder. Bring final volume to 2000 ml with distilled water. Mixed gently to avoid foaming. Transfer to a clean wash bottle and store at 2°-25°C.

ELISA Procedure:

- Add 100 μ l of each, standard, positive control, negative control and sample to appropriate number of wells.
- Add 50 μ l of diluted biotinylated anti-IL-6 to all wells.
- Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for one hour.
- The wells were washed 3 times with diluted washing solution using an automatic washer.

- Add 100µl of Streptavidin-HRP solution into all wells.
- Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for 30 minutes.
- The wells were washed 3 times with diluted washing solution using an automatic washer.
- Add 100µl of ready to use TMB substrate solution into all wells.
- Incubate in the dark for 15 minutes at room temperature. Avoid direct exposure to light by wrapping the plate in aluminium foil. Blue colour developed in the wells.
- Add 100 µl of stopping solution was pipette into each wells and mixed well and the blue colour changed to yellow.
- The absorbance of each well was read within 30 minutes at a wavelength of 450nm with a reference filter of 620nm.

4.9.8. Data analysis:

Calculate absorbance values of standards, controls and samples. Generate a linear standard curve by plotting the average absorbance of each standard on the vertical axis versus the corresponding IL-6 standard concentration on the horizontal axis. The amount of IL-6 in each sample is determined by extrapolating OD values against IL-6 standard concentrations using the standard curve.

5. RESULTS

5.1 The Study Group

A total of 100 neonates (0 to 28 days) who fulfilled the criteria of clinically suspected sepsis were analyzed. This study was conducted at the Department of Microbiology, Tirunelveli Medical College Hospital, Tirunelveli over a period of one year from January 2015 to August 2015.

5.2 Statistical Analysis

All the results obtained were analyzed statistically for their completeness, consistency and accuracy by the parameters like mean and percentages. Kappa value was calculated to measure the degree of agreement between three diagnosis methods- Blood culture with CRP and Blood culture with IL-6. The correlation of serum CRP level and Interleukin-6 level with blood culture for neonatal sepsis was compared statistically and results were analyzed by IBM SPSS Statistics 20. Chi-square test and Fisher Exact test were used in calculating the P-value. The P-Values of less than 0.05 were considered as statistically significant ($P < 0.05$).

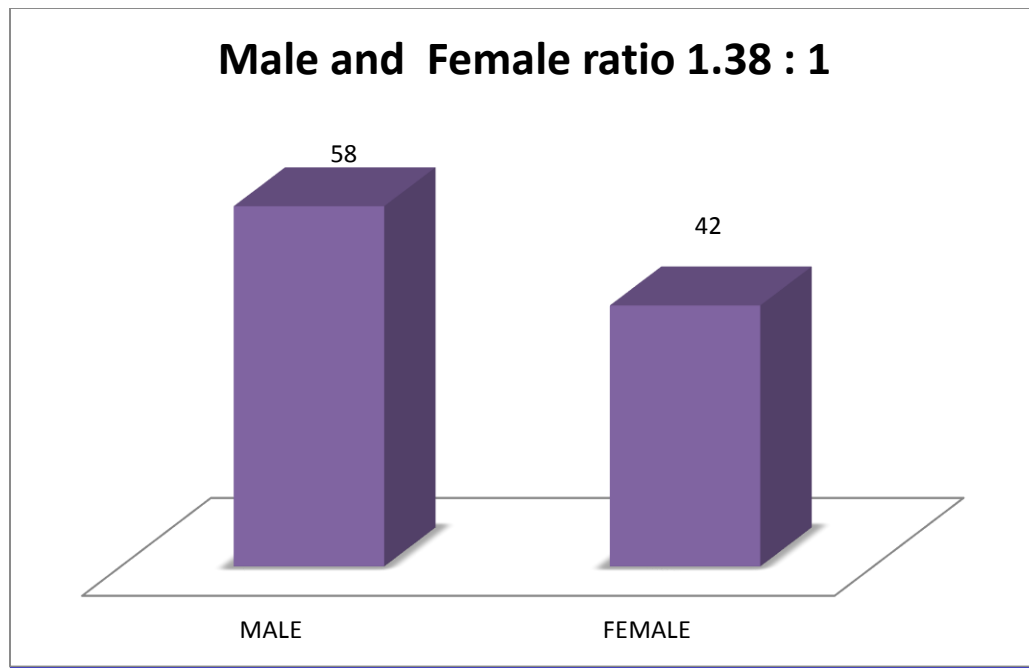
5.3 Result Analysis:

The selected 100 study subjects were analyzed based on age and sex, The results of the analysis are tabulated in Table 1.

Table 1.Age and sex wise distribution in study group

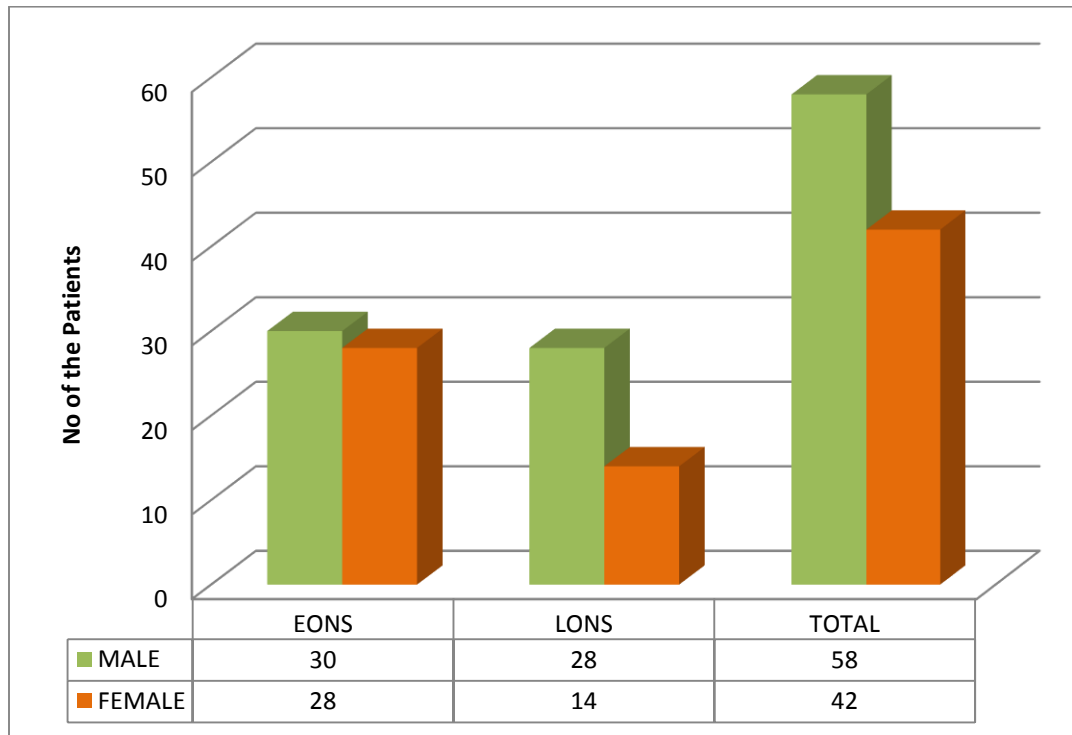
Age (in days)	Male		Female		Total	
	No.	%	No.	%	No.	%
EONS (0-3 days)	30	51.72%	28	66.67%	58	58%
LONS (4-28 days)	28	48.28%	14	33.33%	42	42%
Total	58	100%	42	100%	100	100%

Figure 1:



The above table:1 and figure:1 shows that among the 100 neonates, 58 were male infants and 42 were female infants. Male and female ratio was 1.38:1.

Figure 2. Age and sex wise distribution in study group.



Out of the 100 neonates, 58 (58%) were in the age group of 0-3 days and these neonatal sepsis are called as early onset neonatal sepsis (EONS). In EONS, 30 cases are male and 28 female cases.

The remaining 42 (42%) were in the age group of 4-28 days and these neonatal sepsis are called late onset neonatal sepsis (LONS). In LONS, 28 cases are male and 14 female infants. (Table 1 and Figure 2).

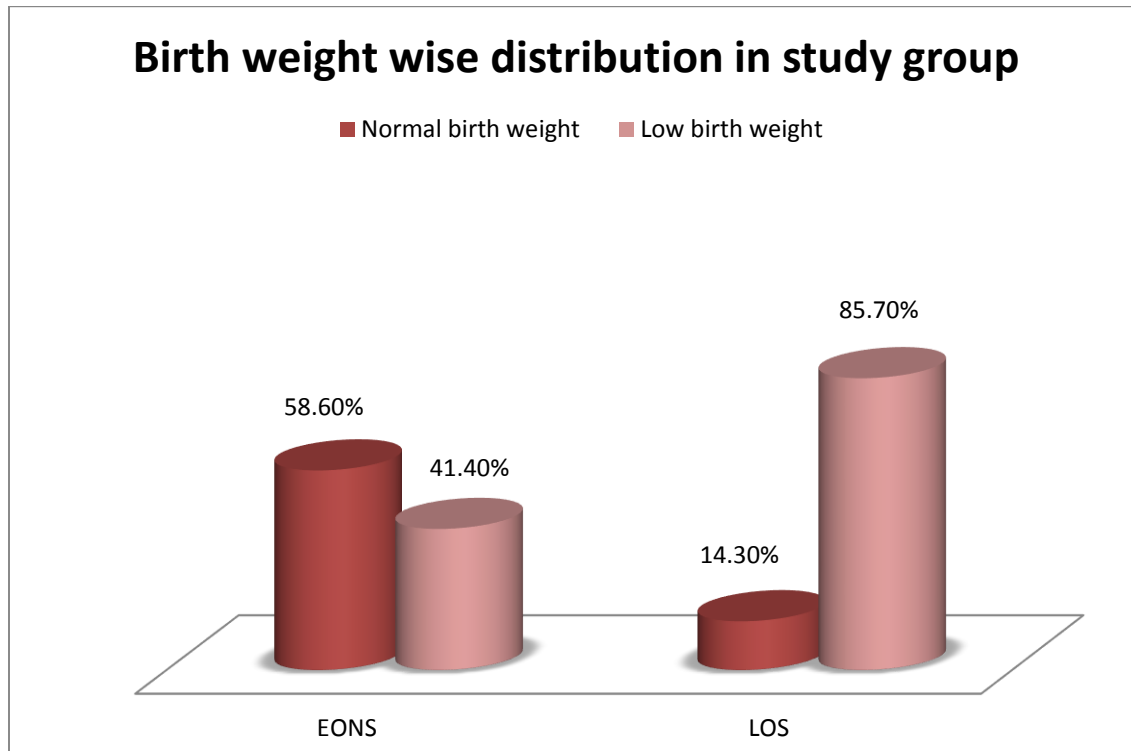
Table: 2

Birth weight wise distribution in study group

Birth weight	EONS		LONS		TOTAL	
	No.	%	No.	%	No.	%
Normal Birth weight (≥ 2.5 Kgs)	34	58.6%	6	14.3%	40	40%
Low Birth weight (< 2.5 Kgs)	24	41.4%	36	85.7%	60	60%
Total	58	100%	42	100%	100	100%

The table : 2 and figure : 3 shows that out of 100 cases studied , 40% of neonates were of normal birth weight (≥ 2500 gms) and 60% of neonates were of low birth weight (<2500 gms).

Figure : 3



Among 58 cases of EONS, 34 (58.6%) cases were normal birth weight and 24 (41.4%) cases were low birth weight.

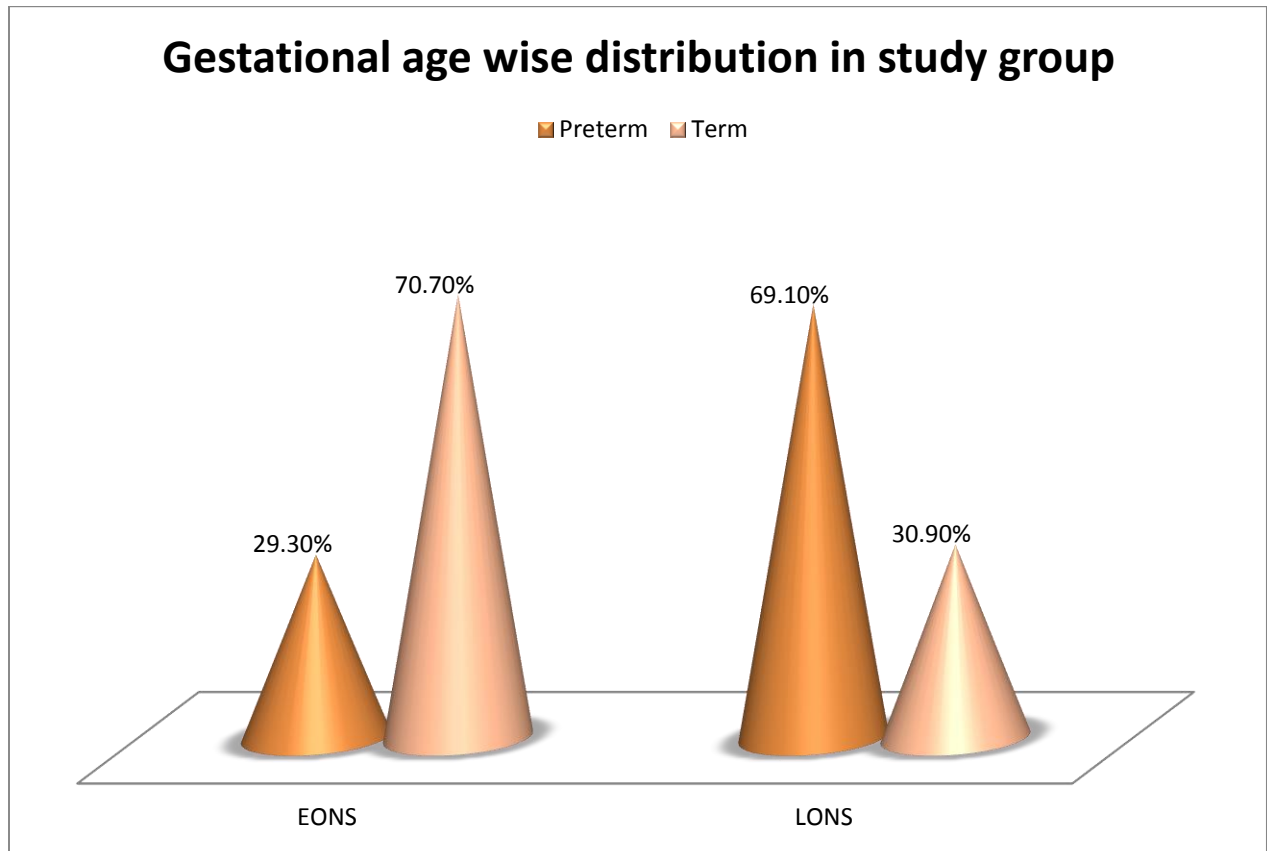
Among 42 cases of LONS, 36 (85.7%) cases were found low birth weight only 6 cases (14.3%) were normal birth weight.

Table : 3 Gestational age wise distribution in study group

Gestational age	EONS		LONS		TOTAL	
	No.	%	No.	%	No.	%
Preterm (< 37 weeks)	17	29.3%	29	69.1%	46	46%
Term (completed 37 weeks)	41	70.7%	13	30.9%	54	54%
Total	58	100%	42	100%	100	100%

The above table :3, figure :4 shows that out of 100 cases studied , 46% of neonates were found to be preterm (< 37 weeks of gestation) and 54% of neonates were found to be term neonates (completed 37 weeks).

Figure :4



Among 58 cases of EONS, 17 (29.3%) cases were found to be preterm and 41 (70.7%) cases were term neonates.

Among 42 cases of LONS, 29 (69.1%) cases were found to be preterm and 13 cases (30.9%) were term neonates.(Table : 3 and Figure:4)

Table : 4 Mode of delivery among study group

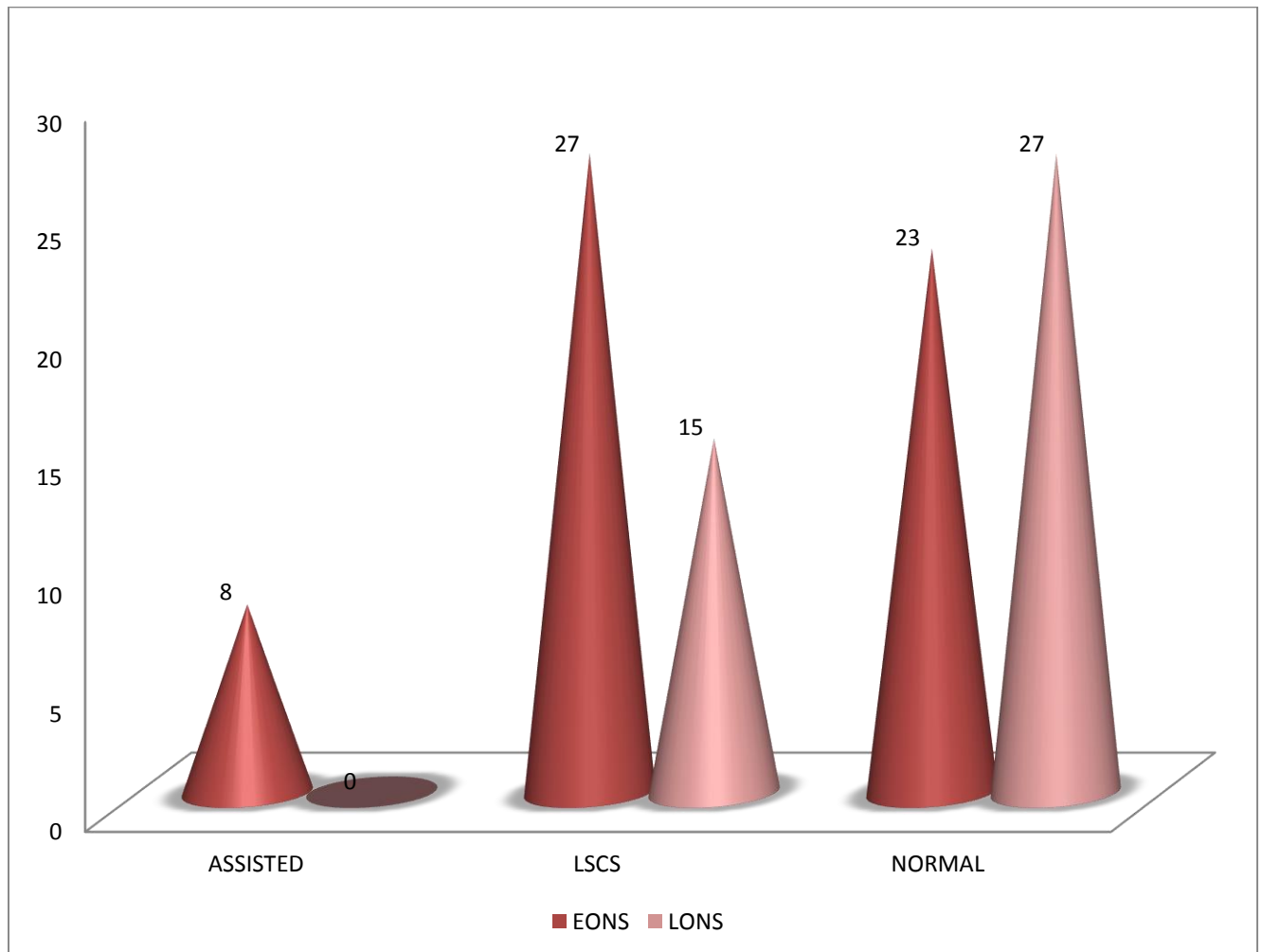
Mode of delivery	EONS		LONS		TOTAL	
	No.	%	No.	%	No.	%
Assisted	8	13.8%	0	0%	8	8%
Lower segment Caesarean Section	27	46.6%	15	35.7%	42	42%
Normal	23	39.6%	27	64.3%	50	50%
Total	58	100%	42	100%	100	100%

The mode of delivery from above table: 4 and figure :5 shows that among the 100 neonates studied, 50 (50%) of the neonates were delivered normally, 42 (42%) of neonates were delivered by Lower segment Caesarean Section (LSCS) and only 8 (8%) of neonates were delivered by assisted vaginal deliveries.

Among 58 cases of EONS, 8 (13.8%) neonates were delivered by assisted vaginal delivery, 27 neonates (46.6%) were delivered by LSCS and 23 neonates (39.6%) were delivered by labour naturale.

Among 42 cases of LONS, 15 neonates (35.7%) were delivered by LSCS and 27 neonates (64.3%) were delivered by labour natural. (Table : 4 and Figure : 5)

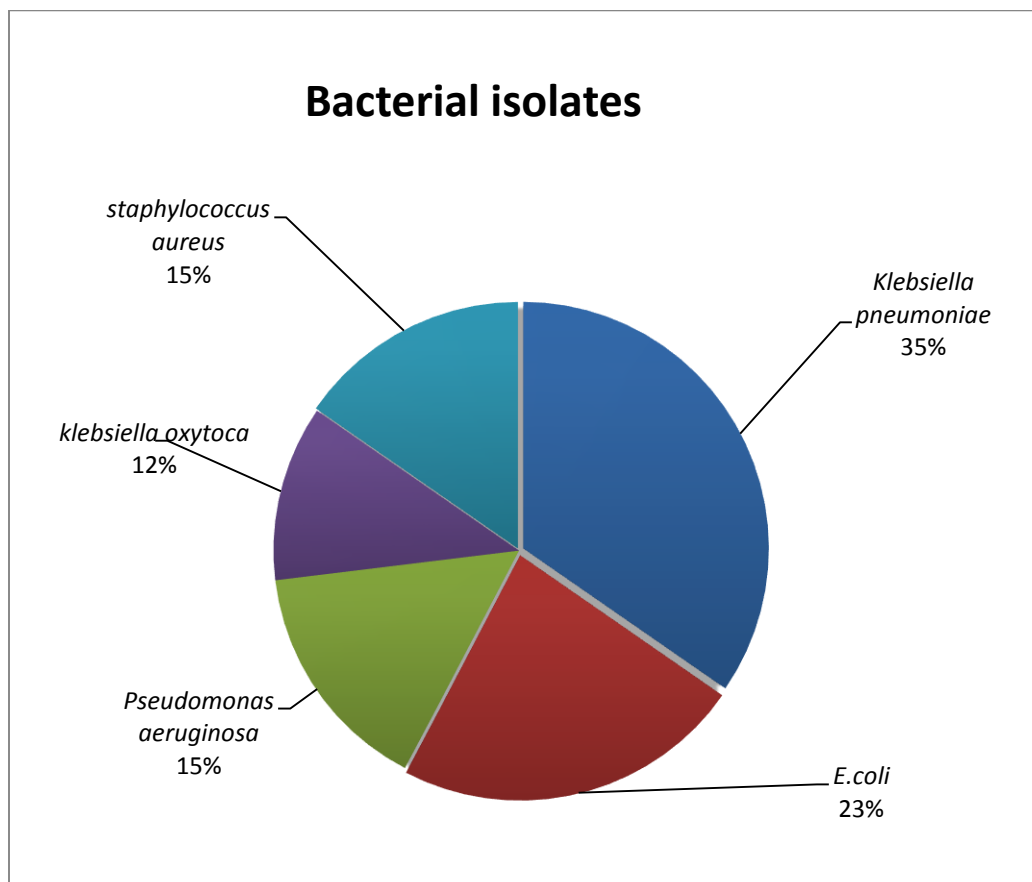
Figure: 5 Mode of delivery among the study group



Isolated bacterial pathogens:

Out of the blood samples collected from 100 participants, positive blood culture was found in 26 cases. The organisms isolated were *Klebsiella pneumoniae* in 9 neonates (35%), *Escherichiae coli* in 6 neonates (23%), *Pseudomonas aeruginosa* in 4 neonates (14%), *Klebsiella oxytoca* in 3 neoates (12%), and *Staphylococcus aureus* in 4 neonates (15%). *Klebsiella spp* was found to be a most common organism in both early and late onset sepsis. (Figure : 6)

Figure: 6 Distribution of the isolated bacterial pathogens



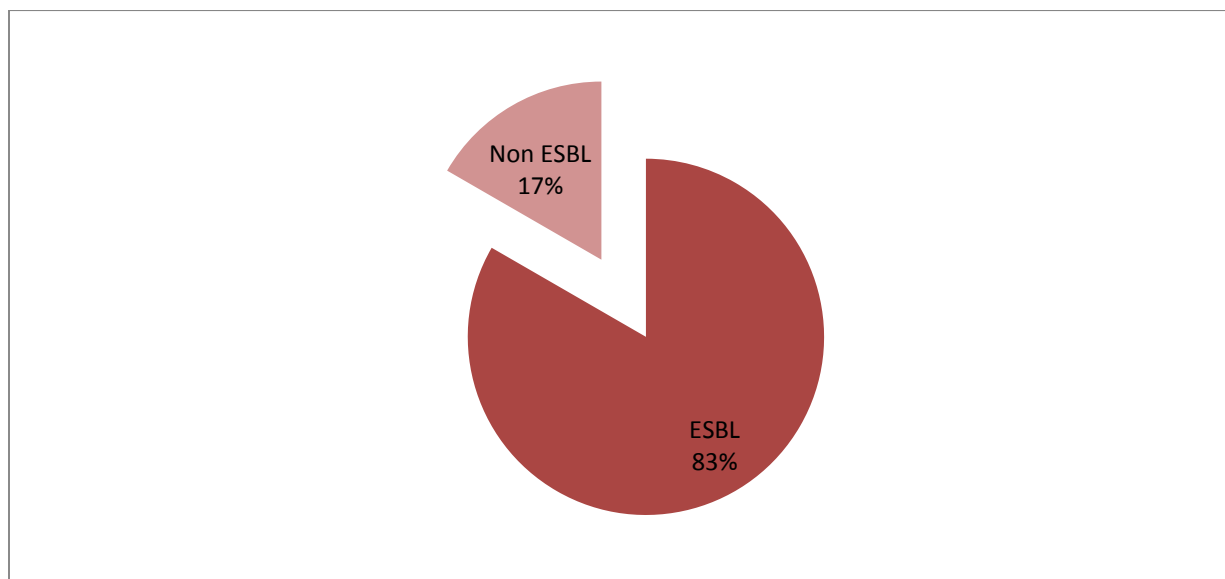
Antimicrobial sensitivity pattern of the isolated organism:

Out of 9 *Klebsiella pneumoniae* isolates, 8 were resistant to third generation cephalosporins with Extended Spectrum Beta Lactamase (ESBL) phenotype. It contributes 89 % among of *Klebsiella pneumoniae* isolates. Only one isolate was sensitive to third generation cephalosporin group.

In three *Klebsiella oxytoca* isolates, two isolates were found to be resistant to third generation cephalosporin with ESBL phenotype and remaining one isolate was sensitive to third generation cephalosporin

Figure: 7

ESBL among *Klebsiella spp*



Among 12 isolates of *Klebsiella spp*, 10 isolates (83%) were resistant to third generation cephalosporin with ESBL phenotype. But all 12 *Klebsiella spp* isolates were sensitive to imipenem. (Figure :7)

Out of 6 *Escherichiae coli* isolates, five (80%) were sensitive to third generation cephalosporin. Only one case (20%) was resistant to third generation cephalosporin with ESBL phenotype. But all 22 gram negative isolates were sensitive to imipenem and cefperazone-sulbactam. Among four *Staphylococcus aureus*, 3 isolates (75%) were methicillin resistant strain (MRSA).

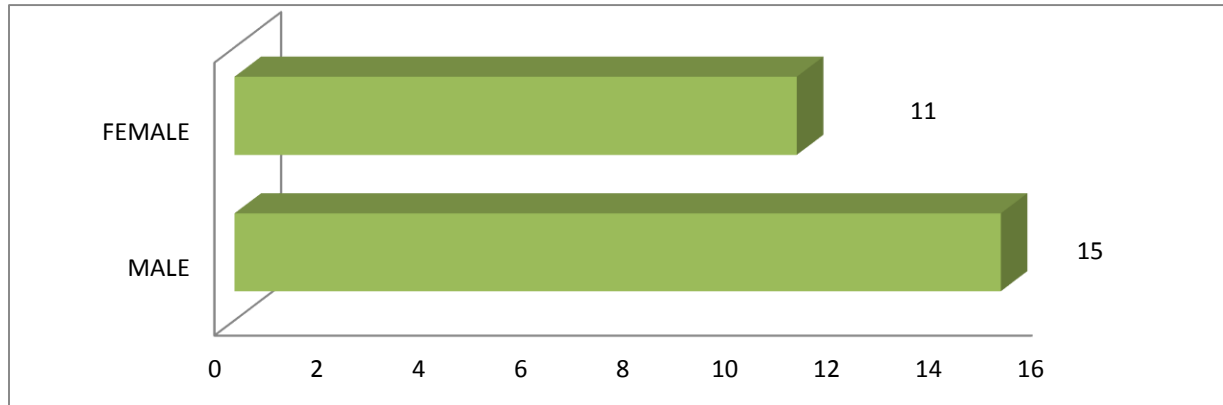
Out of 26 bacterial isolates, 21 isolates (81%) were sensitive to amikacin and remaining 5 (19%) were resistant to amikacin. Among 26 bacterial isolates, 20 isolates (76.9%) were sensitive to gentamicin and 6 (23.1%) were resistant to gentamicin. Out of 26 bacterial isolates, 21 isolates (81%) were sensitive to ciprofloxacin and 12 isolates (46.1%) were sensitive to cotrimoxazole.

Table: 5 **Sensitivity pattern of all 26 bacterial isolates**

Antibiotics	<i>K.pneumoniae</i>		<i>K.oxytoca</i>		<i>E.coli</i>		<i>P.aerugenosa</i>		<i>S.aureus</i>	
	n=9	%	n=3	%	n=6	%	n=4	%	n=4	%
Gentamicin	7	78	2	67	5	83	2	50	4	100
Amikacin	7	78	2	67	5	83	3	75	4	100
Cefotaxime	2	22	1	33	5	67	1	25	-	-
Ceftriaxone	2	22	1	33	5	67	1	25	-	-
Ceftazidime	2	22	1	33	5	67	1	25	-	-
Co trimoxazole	5	56	1	33	4	67	0	0	2	50
Ciprofloxacin	7	78	2	67	5	83	3	75	4	100
Imipenem	9	100	3	100	6	100	4	100	-	-
Piperacillin-Tazobactam	9	100	3	100	6	100	4	100	-	-
Ampicillin	-	-	-	-	-	-	-	-	2	50
Cefoxitin	-	-	-	-	-	-	-	-	2	50
Erythromycin	-	-	-	-	-	-	-	-	3	75
Clindamycin	-	-	-	-	-	-	-	-	3	75
Vancomycin	-	-	-	-	-	-	-	-	4	100

Figure: 8

Sex wise distribution among blood culture positive cases



Above figure: 8 show among 26 blood culture positive cases, 15 (57.7%) cases were male neonates and it contributes 57.7% of total blood culture positive cases. Another 11 cases were female neonates and it contributes 42.3% of total blood culture positive cases. There was no statistically significant difference between sex wise and culture positive cases. ('p' value is >0.05)

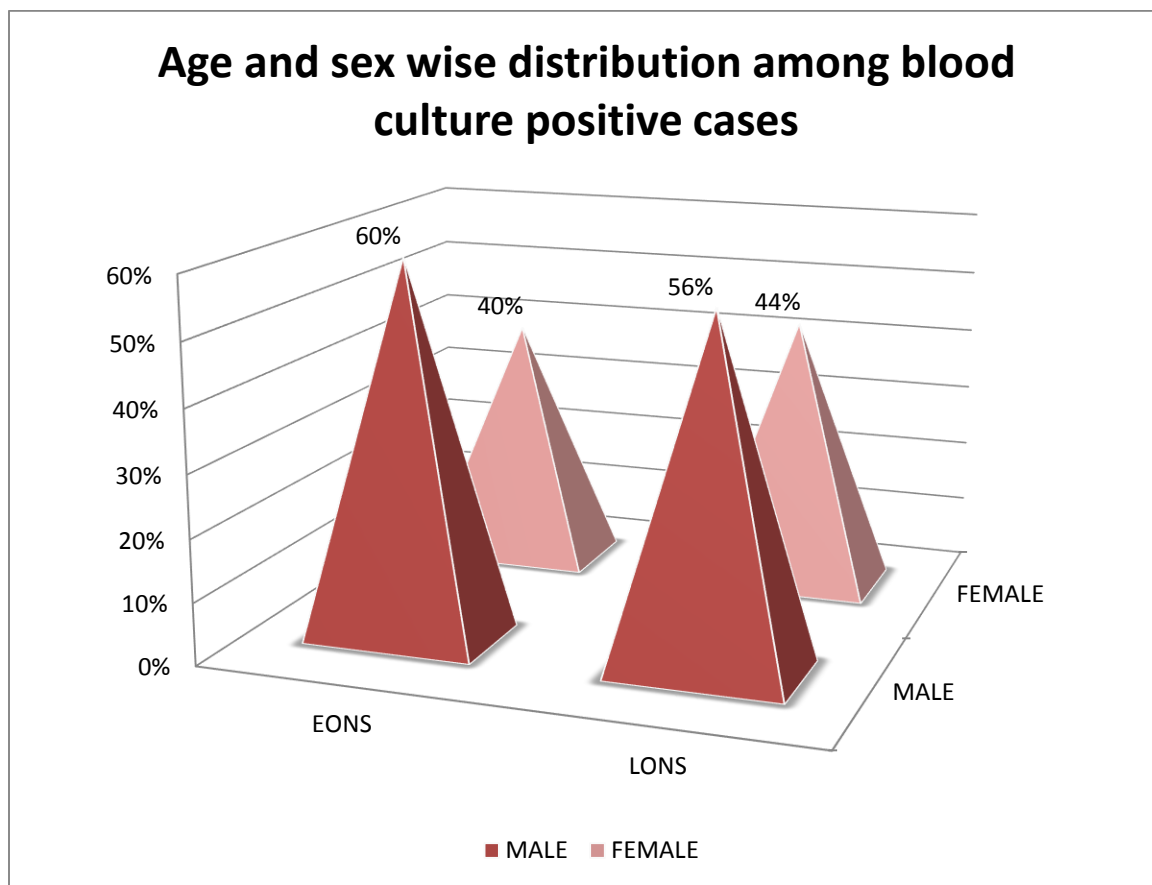
Table: 6

Age wise distribution among blood culture positive cases

SEX	EONS		LONS		TOTAL	
	No.	%	No.	%	No.	%
Male	9	60%	6	40%	15	100%
Female	6	56%	5	44%	11	100%

Out of 26 blood culture positive cases, 15 cases were in the age group of 0-3 days and it contributes 57.7% of total culture positive cases. Remaining 11 cases were in the age group of 4-28 days and it contributes 42.3% of total blood culture positive cases. (Table : 6 and Figure :9)

Figure: 9



Among 15 EONS cases, 9 cases (60%) were male neonates and 6 cases (40%) were female infants. Among 11 LONS cases, 6 cases (56%) were male newborns and 5 cases (44%) were female newborns. (Table : 6 and Figure :9)

Table: 7

Gestational age wise distribution among blood culture positive cases

Gestational Age	EONS	LONS	TOTAL
Preterm	9	11	20
Term	6	0	6
Total	15	11	26

Above table:7 and figure: 10, shows that out of 26 blood culture positive cases, 20 cases (76.7%) were preterm babies and remaining only 6 cases (23.30) were term babies.

Among 20 preterm babies, 11 cases belong to late onset neonatal sepsis category.

In 15 cases of EONS, 9 cases were preterm and another 6 cases were term newborns. All 11 cases of LONS are preterm babies. There was a statistically significant association between gestational age and neonatal sepsis cases. ('p' value is 0.024).

Figure: 10

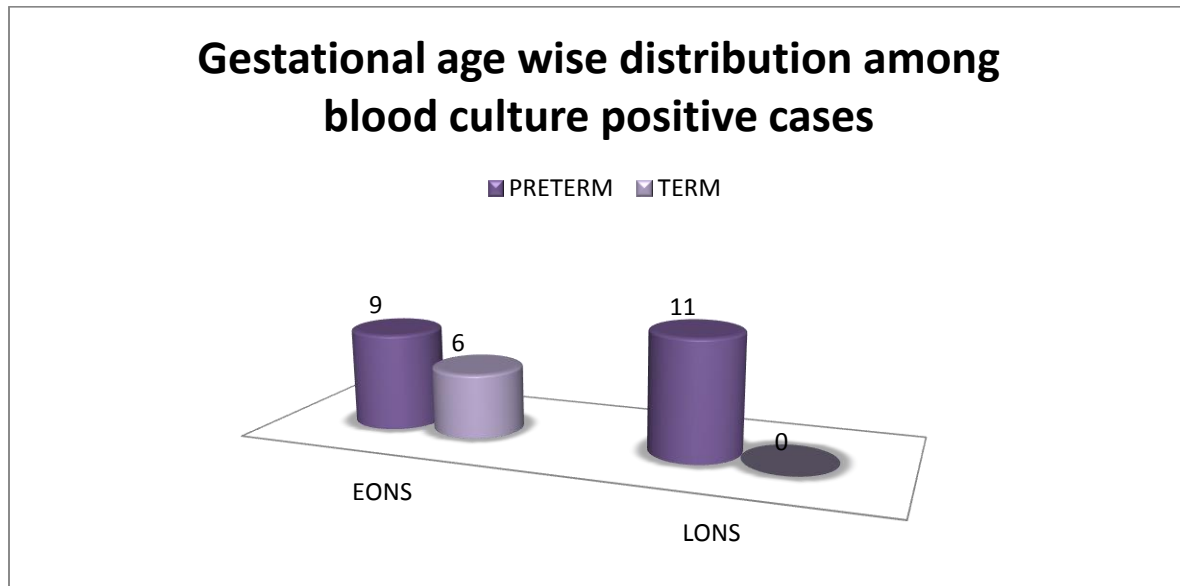


Table : 8

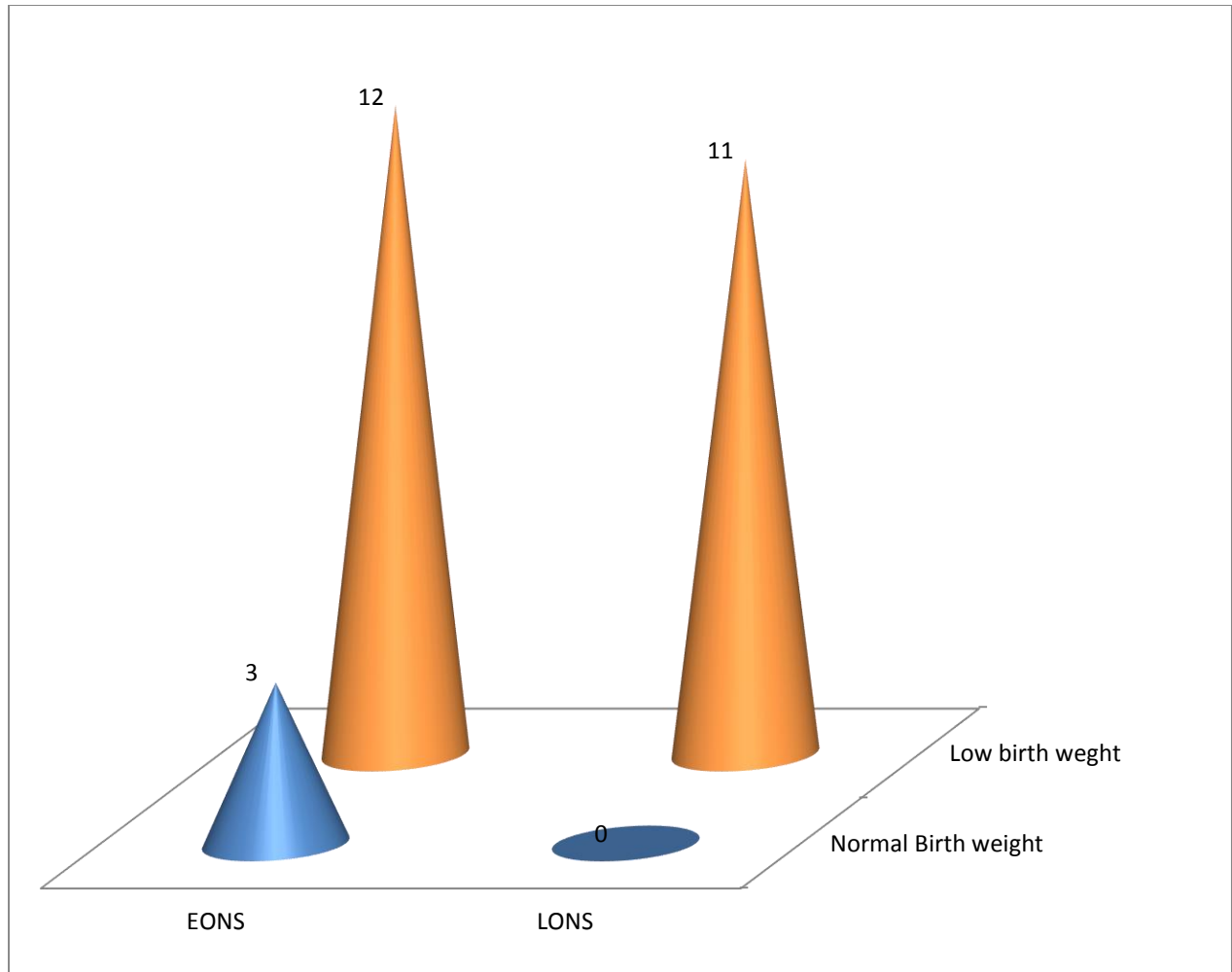
Association of birth weight in positive cases

Birth Weight	EONS	LONS	TOTAL	%
Normal (≥ 2500 Gms)	3	0	3	11.50%
Low (< 2500 Gms)	12	11	23	86.50%

The above table : 8 and figure: 11, shows that 23 (86.5%) blood culture positive cases were low birth weight (less than 2500 gm) neonates. Only 3 cases (11.5%) were having birth weight above 2500 gms.

Figure: 11

Birth weight wise distribution wise among positive cases



All 11 (100%) cases of LONS are low birth weight babies. In 15 of EONS, 12 (80%) cases were having low birth weight and remaining three (20%) cases were having normal birth weight. (Table:8 and Figure:11) There was no statistically significant difference between birth weight and culture positive cases. ('p' value is >0.05)

Table: 9

Mode of delivery wise distribution among positive cases

MODE OF DELIVERY	EONS		LONS		TOTAL	
	No.	%	No.	%	No.	%
ASSISTED	2	13.33%	0	0%	2	7.69%
LSCS	5	33.33%	3	27.27%	8	30.77%
NORMAL	8	55.33%	8	72.73%	16	61.54%
TOTAL	15	100%	11	100%	26	100%

Out of 26 blood culture positive cases, 16 cases (61.54%) delivered by labour natural, 8 cases (30.76%) delivered by LSCS and remaining two new born babies delivered by assisted vaginal delivery.

In 15 cases of EONS, 8 cases (55.33%) delivered by labour natural, 5 cases (33.33%) delivered by LSCS and remaining two new born babies were delivered by assisted vaginal delivery.

In 11 cases of LONS, 8 cases (72.73%) delivered by labour natural and 3 cases (27.27%) delivered by LSCS. There was no statistically significant difference between mode of delivery and culture positive cases. ('p' value is >0.05)(Table:9 and Figure :12)

Figure:12

Mode of delivery wise distribution among positive cases

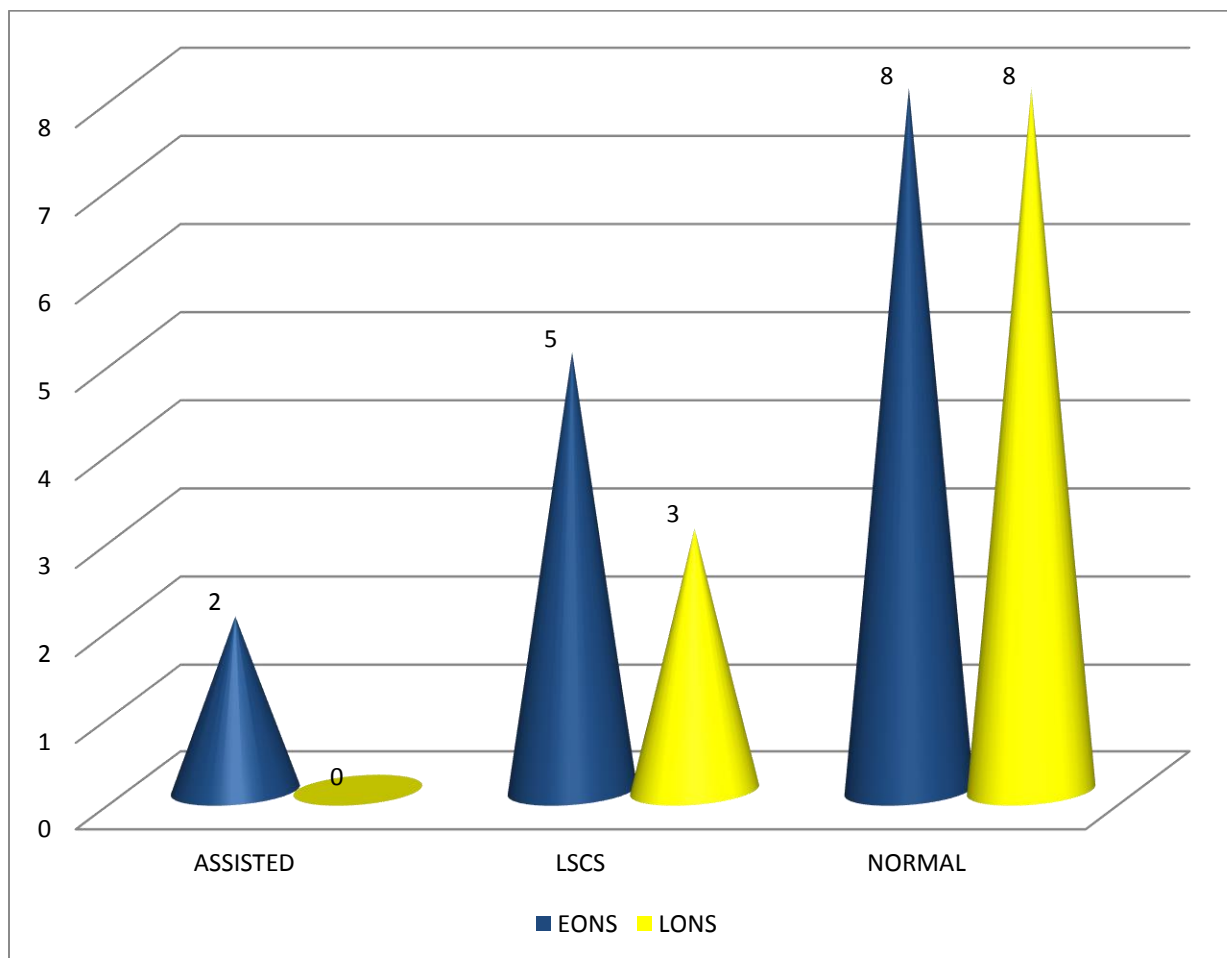


Table:10

Neonatal mortality

	EONS		LONS		Total	
Death	n=58	%	n=42	%	n=100	%
	8	13.8	5	11.9	13	13%

The neonatal mortality rate of present study was 13% (13 cases). Among these, EONS cases were 8 and LONS cases were 5 cases. Out of 8 EONS cases, 5 cases were blood culture positive and 3 cases were blood culture negative. Out of 5 LONS cases, 4 cases were blood culture positive and one case was culture negative.

C-reactive protein results in study group:

Out of 100 clinically suspected neonatal cases, 40 neonates were C-reactive protein test positive. Out of the 40 positive cases 26 neonates (65%) were EONS and 14 cases (35%) were of LONS. (Figure : 13 and Table : 11)

Figure:13

CRP positive cases in study group

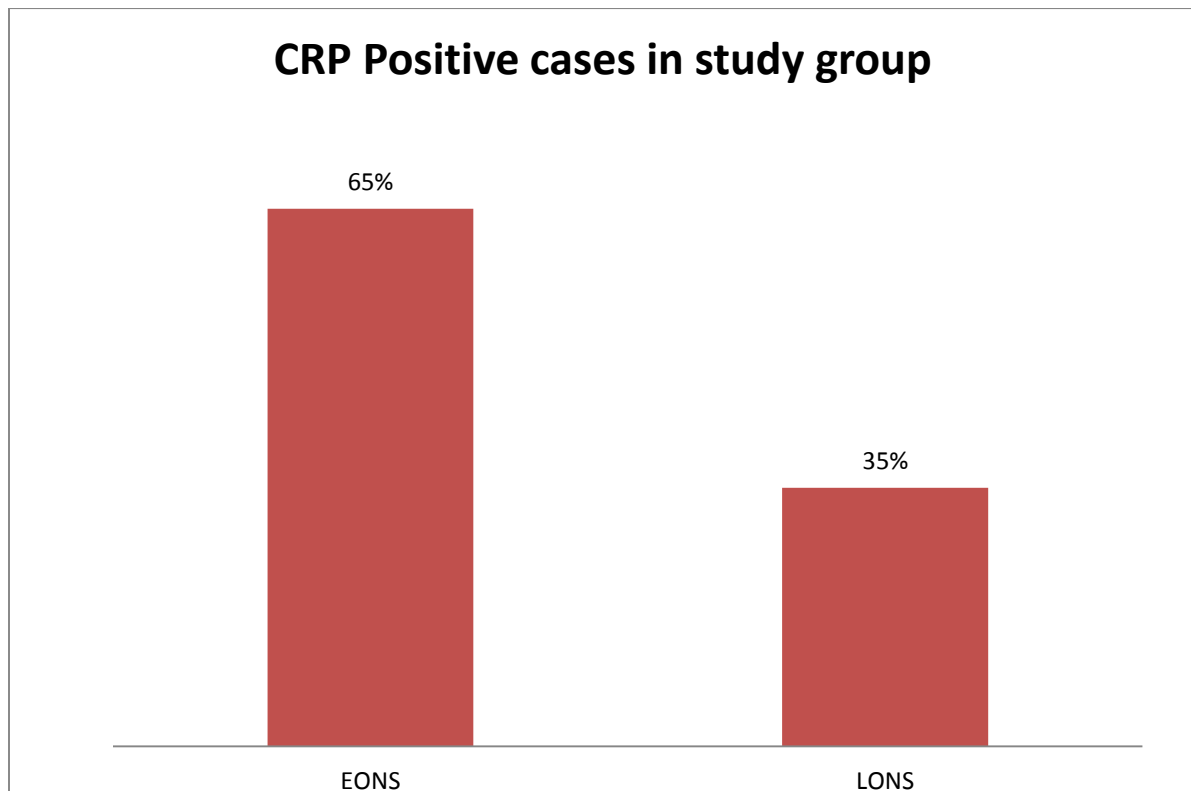


Table: 11

Association between blood culture results and CRP results

CRP	Blood Culture		TOTAL
	POSITIVE	Negative	
POSITIVE	14	26	40
NEGATIVE	12	48	60
TOTAL	26	74	100

Detection of CRP by latex agglutination method was evaluated for its sensitivity and specificity against blood culture as reference test.

$$\text{Sensitivity} = \frac{TP}{TP+FN} = \frac{14}{26} \times 100 = 53.84\%$$

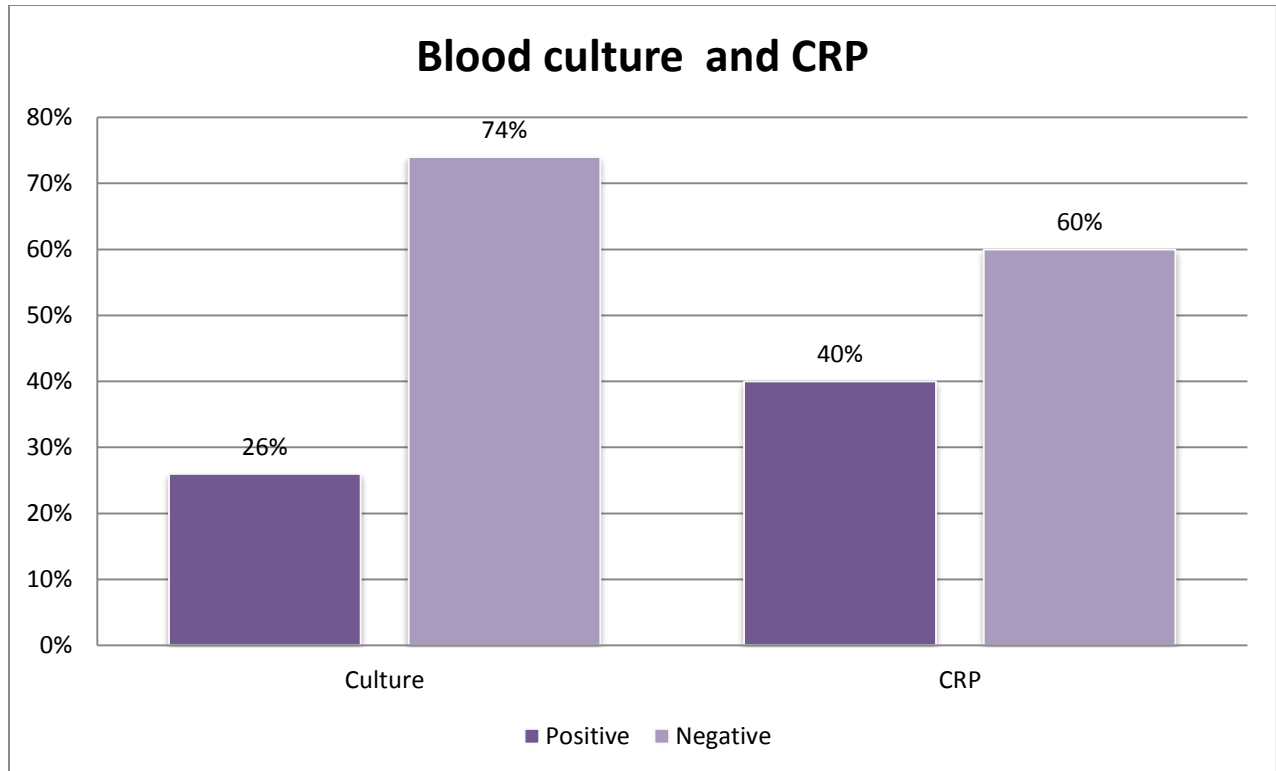
$$\text{Specificity} = \frac{TN}{TN+FP} = \frac{48}{74} \times 100 = 64.86\%$$

$$\text{Positive predictive value} = \frac{TP}{TP+FP} = \frac{14}{40} \times 100 = 35\%$$

$$\text{Negative predictive value} = \frac{TN}{TN+FN} = \frac{48}{60} \times 100 = 80\%.$$

Figure: 14

Association between blood culture results and CRP results



From the above table : 11 and figure : 14, sensitivity of CRP latex agglutination test was 53.84% when evaluated against culture, a reference test. Specificity of CRP latex agglutination test was 64.86% compared to culture and positive and negative predictive value were 35% and 80% respectively. According to McNemer's test p value was 0.034. It was found to be statistically significant.

Table:12

CRP semi quantitative Assay

CRP	No. Of cases
>6 mg/L	27
>12 mg/L	1
>24mg/L	5
>48mg/L	7
Negative	60
Total	100

Above table:12 Shows, among 40 CRP positive cases, 7 cases were under serum CRP level more than 48mg/L category, 5 cases were CRP level in between 24 - 48mg/L category, one case was CRP level in between 12-24mg/L category and 26 cases were CRP level under 6 -12 mg/L category.

Out of 40 CRP positive cases, 14 cases were blood culture positive. Among 14 culture positive cases, 13 cases due to gram negative organisms (high titre of serum CRP more than 12 mg/L) and one case due to gram positive organism(low titre of serum CRP more than 6 mg/L)

Serum Interleukin-6 results in study group:

Out of 100 clinically suspected neonatal cases, 39 neonates were Interleukin-6 positive. On the 39 positive cases 23 (58.97%) neonates were EONS and 16 (41.03%) cases were of LONS. (Figure: 15)

Figure:15

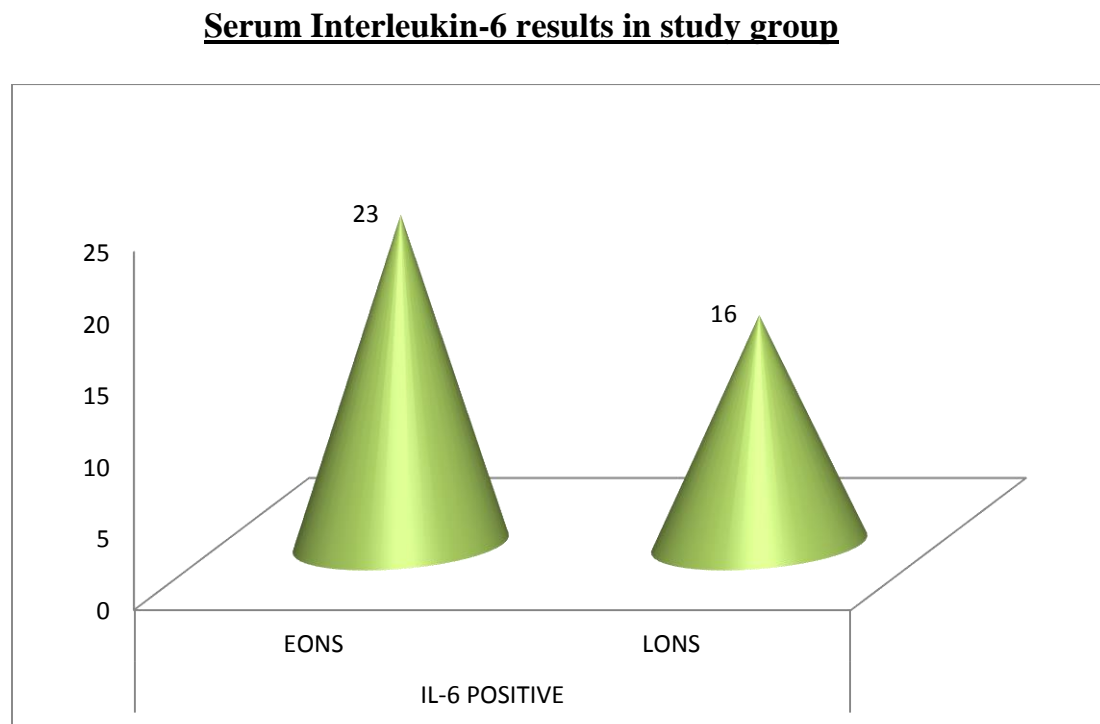


Table: 13

Evaluation of Interleukin-6 with blood culture

IL-6	Blood Culture		TOTAL
	POSITIVE	NEGATIVE	
POSITIVE	25	14	39
NEGATIVE	1	60	61
TOTAL	26	74	100

Detection of serum Interleukin-6 level by ELISA method was evaluated for its sensitivity and specificity against blood culture, as reference test.

$$\text{Sensitivity} = \frac{TP}{TP+FN} = \frac{25}{26} \times 100 = 96.15\%$$

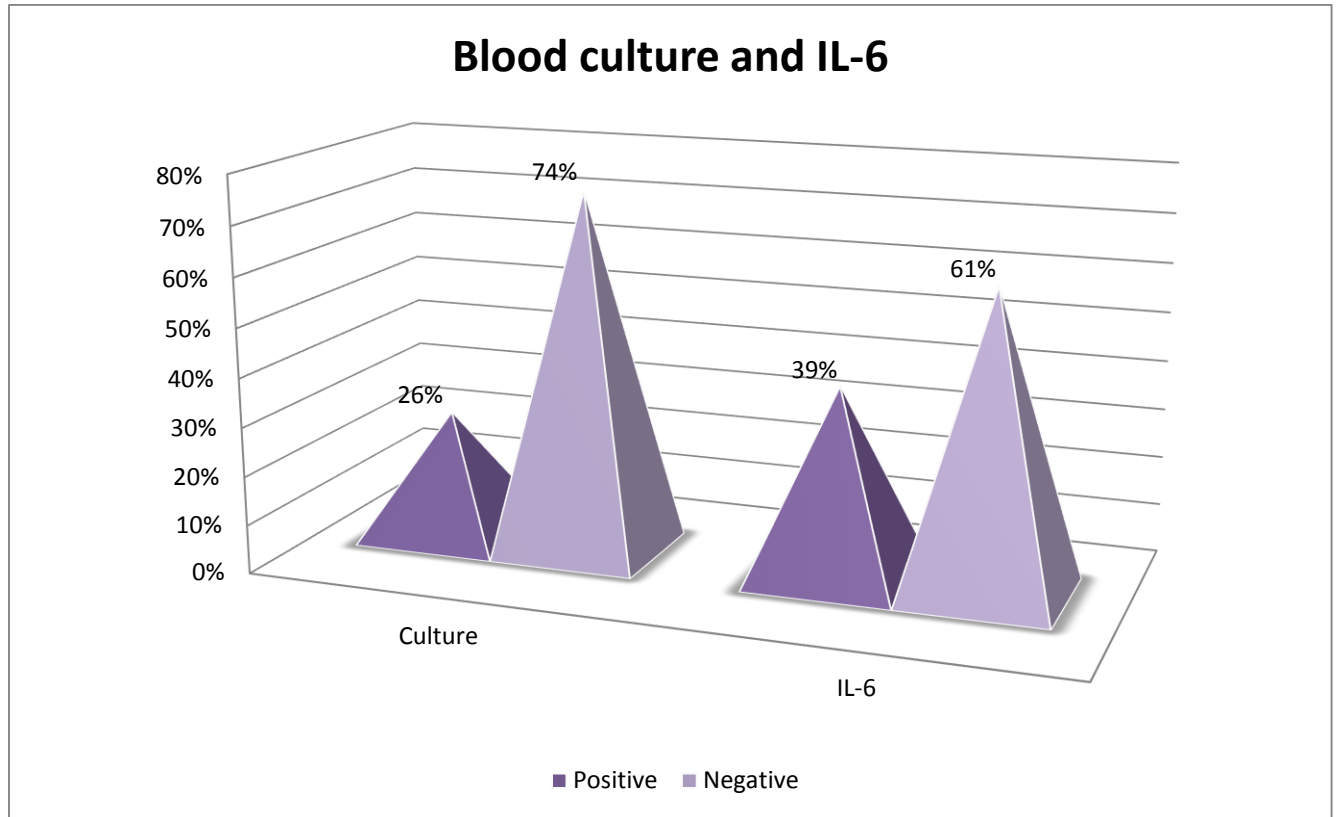
$$\text{Specificity} = \frac{TN}{TN+FP} = \frac{60}{74} \times 100 = 81.08\%$$

$$\text{Positive predictive value} = \frac{TP}{TP+FP} = \frac{25}{39} \times 100 = 64.10\%$$

$$\text{Negative predictive value} = \frac{TN}{TN+FN} = \frac{60}{61} \times 100 = 98.36\%.$$

Figure: 16

Evaluation of Interleukin-6 with blood culture



From the above table:13 and figure : 16, sensitivity of detection of IL-6 by ELISA method was 96.15% when evaluated against culture , a reference test. Specificity of this test was 81.08% compared to culture and positive and negative predictive value were 64.1% and 98.36% respectively. According to Mc Nemer's test, 'p value' was 0.001. It was found to be statistically significant.

Table:14**IL-6 Quantitative Assay**

IL-6 assay by ELISA	No. Of cases
Highly Positive (100-200pg/ml)	20
Moderately Positive (50-100pg/ml)	5
Weakly Positive (< 50- 6.25pg/ml)	14
Negative <6.25pg/ml	61
Total cases	100

Out of 39 IL-6 positive cases, 20 cases were under highly positive category (IL-6 level 100-200pg/ml, OD value 1.21-2.34), 5 cases were under moderately positive category (IL-6 level 50-100pg/ml, OD value 0.76-1.20) and 14 cases were weakly positive category (IL-6 level 6.25 -50 pg/ml, OD value 0.15-0.75).

Among 39 IL-6 positive cases, 25 cases were culture positive (22 cases due to gram negative organisms and 3 cases due to gram positive organism). Among 22 gram negative neonatal cases, 20 cases have high titre of IL-6 and remaining 2 gram negative neonatal sepsis cases have moderately positive titre of IL-6. All the three gram positive neonatal sepsis cases have moderately positive titre of IL-6.

6. DISCUSSION

Neonatal sepsis is defined as a clinical syndrome of bacteremia with systemic signs and symptoms of infection in the first 4 weeks of life. It encompasses various systemic infections of the newborn such as septicemia, meningitis, pneumonia, arthritis, osteomyelitis and urinary tract infections. Superficial infections like conjunctivitis and oral thrush are not usually included under neonatal sepsis. The aim of the present study was to detect the bacteriological profile of neonatal sepsis and antibiotic susceptibility pattern of the isolates and also to determine the value of C-reactive protein and Interleukin-6 in establishing the diagnosis of neonatal sepsis.

6.1 Age and Sex wise distribution in study group:

The present study shows that out of 100 clinically suspected neonatal sepsis cases, male neonates were found to be predominant than female neonates in the ratio of 1.38 : 1.

Male predominance was also observed in similar studies conducted by YR Khinchi AK *et al* and Bambala Puthattayil Zakariya *et al*. This male predominance may be due to X-linked immune regulatory gene factor contributing to host's susceptibility to infections in male neonates and also due to the prevalent custom of taking male babies preferentially to hospital.^{13,28,51}

Among 100 study cases, 58 % of cases were under the age group of 0 – 3 days (<72 hours) and remaining 42 % of cases were under the age group of 4-28 days. Flora Chacha *et al* and Sucila Thangam *et al* also reported in a similar study where early onset neonatal sepsis is more prevalent than late onset neonatal sepsis.^{15,16}

In contrast there was a high prevalence of LONS (60%) in the study conducted by Neema Kayange *et al* in Tanzania among 300 clinically suspected neonatal sepsis cases.¹⁷

6.2 Birth weight wise distribution in study group:

The current study done with 100 neonates showed 58 neonates had EONS and 42 had LONS. Among the reported EONS, 58.6% were of normal birth weight and 41.4% were of low birth weight. Among the reported LONS, 14.3% were of normal birth weight and 85.7% were of low birth weight.

Similar result had been reported in a comparative study by Rabindra N Misra *et al* which showed that out of 75 culture positive cases, 75% of late onset sepsis occurred in low birth weight babies.¹⁹ A study by Jun-Ho Wu, *et al* found that more LONS cases were present in preterm and low birth weight babies. The immuno compromised state of low birth weight and preterm babies may be the cause of more infection in these group.⁸²

6.3 Gestational age wise distribution in study group :

The present study shows that out of 58 EONS cases reported, 17 cases (29.3%) come under preterm category and 41 cases (70.7%) come under term category. Among 42 LONS cases reported, 29 cases (69.1%) come under preterm category and 13 cases (30.9%) come under term category.

In similar study by Bambala Puthattayil Zakariya *et al* and Subhranshu Sekhar Kar *et al* observed increased prevalence of neonatal sepsis among preterm infants when compared with term neonates.^{28,51}

This increased susceptibility of neonatal sepsis among preterm cases could be attributed to inherent deficiency of both humoral and cellular immunity during the first week of life.²⁸

6.4 Distribution of isolated bacterial pathogens among study group:

The current study shows that among 100 suspected sepsis cases, blood culture was positive in 26 cases (26%).

Similar positivity percentage of blood culture had been reported in the study by Sucila Thangam *et al* during April – September 2010 which revealed 28% of positive blood culture in 50 samples.¹⁶ Another study by Shrestha R K *et al* in Nepal medical college, Kathmandu, during the period of July 2011 to January 2012 reported similar range results of 30.8% of positive blood culture.⁵²

In contrast there was a high positivity report of blood culture in the study done by Rajalakshmi Vishwanathan *et al* in Kolkatta during March 2009 –August 2010 which revealed 46.3% of positive blood culture in 216 samples.²⁷ In contrast low positivity of blood culture had been reported in studies by Subhranshu Sekar Kar *et al* in Bhubaneswar during the study period (2007–2010) which revealed 16.2% of positive blood culture in 120 samples.⁵¹

Reasons for comparative variability in blood culture positivity of various studies might be due to administration of prior antibiotics from primary centre, infection with anaerobes and effective control in spread of hospital acquired infection.^{27,28}

Another reason attributed to the variable positive result may depend on the mode of test employed for culture. Commonly used conventional test is time consuming and its yield is of significantly low sensitivity when compared to fully automated system which is less time consuming and has the benefit of continuous monitoring with high sensitivity.²⁸

Out of the 26 isolates, *Klebsiella pneumoniae* were 9 (35%), *Escherichiae coli* were 6 (23%), *Pseudomonas aeruginosa* were 4 (15%), *Klebsiella oxytoca* were 3 (12%) and *Staphylococcus aureus* were 4 (15%). *Klebsiella spp* was found to be the most common organism in both early and late onset sepsis.

In similar studies by Bambala Puthattayil Zakariya *et al*, Neema Kayange *et al* and Iregu KC *et al* results had been reported that gram negative bacterial infection were more prevalent than the gram positive bacteria. Among gram negative organism, *Klebsiella pneumoniae* were predominant isolates.^{17,28,29}

In contrast in a study conducted by Shrestha R K *et al* in Nepal medical college, Kathmandu, during the period of July 2011 to January 2012, *Staphylococcus aureus* (56.8%) had been reported as main etiological agent in neonatal sepsis and followed by *Klebsiella pneumoniae* (21.7%), *Pseudomonas aeruginosa* (13.4%) and others. Highest prevalence of *Staphylococcus aureus* could be attributed to nosocomial infection from carrier in relatives and hospital staffs.⁵²

6.5 Antibiotics susceptibility pattern of isolated pathogens:

The present study shows that among 12 *Klebsiella spp*, 9 isolates were *Klebsiella pneumoniae* species and remaining 3 isolates were *Klebsiella oxytoca*.

Out of 12 isolates of *Klebsiella spp*, 83% (10 isolates) were resistant to third generation cephalosporin with ESBL phenotype.

A similar study done by Iregu KC *et al* found that 87% of *Klebsiella pneumoniae* isolates were found to be ESBL producers. Another similar study done in North India by Kaistha N *et al* found that 88% gram negative isolates were resistant to third generation cephalosporin.^{29,30}

Based on study done by Bambala Puthattayil Zakariya *et al* in Jawaharlal Nehru Institute of Post graduate, Medical Education and Research Puducherry, found that 32% of *Klebsiella pneumoniae* isolates were found to be ESBL producers. It was in contrast to present study.²⁸

Klebsiella pneumoniae is one of most common pathogen profoundly found in neonatal intensive care ward environment and various equipment in neonatal ward. So *Klebsiella pneumonia* causes neonatal septicemia outbreaks in NICU. The third

generation cephalosporin are commonly used empirically in all cases of clinically suspected sepsis in present study centre. It could be the main cause for increased incidence of ESBL in the present study.

But all 12 isolates of *Klebsiella spp* are sensitive to imipenem. Similar result was obtained in the study done by Neema Kayange *et al* with about 95% of *Klebsiella spp* sensitive to imipenem. But this drug is costly and not freely available in all health care centers in developing countries.¹⁷

The present study shows that among four *Pseudomonas aeruginosa*, 2 isolates (50%) were resistant to gentamicin, 3 isolates (75%) were resistant to third generation cephalosporin and one isolate was resistant to ciprofloxacin. But all four isolates were sensitive to imipenem and Piperacillin-Tazobactam. This finding similar to study of Bambala Puthattayil Zakariya *et al*.²⁸

The current study shows that among four *Staphylococcus aureus*, 3 isolates (75%) were resistant to methicillin (MRSA). In a similar observation made by Ramesh Bhat Y *et al* showed that 79% of *staphylococcus aureus* isolates were MRSA.²²

A study done by Neema Kayange *et al* found that among 149 culture positive cases, 32 were *Staphylococcus aureus*. Out of that 32 *Staphylococcus aureus* isolates 28% were resistant to Methicillin. It was in contrast to the present study.¹⁷

Increased prevalence of MRSA may be due to healthy carrier in hospital staffs and relatives.

The present study shows, among 26 bacterial isolates, 21 isolates (81%) were sensitive to amikacin. This study is in accordance with the study conducted by Kuruvilla

*et al.*²³ The current study also shows, 21 isolates (81%) were sensitive to ciprofloxacin. This finding was also similar with studies of Neema Kayange *et al* and Boma AW *et al.*^{26,88} Increased sensitivity of ciprofloxacin may be due to the practice of that drug not being routinely prescribed to paediatric age group. But according to studies of Omokhodion *et al* and Boma AW *et al* revealed that quinolone group was successfully and safely useful in neonatal sepsis.^{88,89}

6.6 Neo natal mortality:

The present study shows that 13% (13 cases) of neonatal mortality among study group. This mortality rate was low compared to similar studies in Tamilnadu and India.^{23,42} This declining trend of neonatal mortality attributed to effective health care management system, strict implementation of infection control protocols in hospital and availability of good infra structures and laboratory facilities which all in turn helps in early diagnosis and prompt management of neonatal infection.

6.7 Prevention of drug resistance:

The emergence of resistance to the antibiotics should be constantly monitored so that the antibiotic cycling policies and the occurrence of the newer resistance pattern in the hospital can be determined. There is also a necessity to highlight on the rational use of antibiotics and strict adherence to the concept of reserve drugs to reduce the inappropriate use of the available antibiotics.

Various strategies like hand washing, environmental decontamination, education to the health care professionals and continuous surveillance will be helpful in attaining a

goal of low level of infection with the multidrug resistant pathogens. The hospital infection control guidelines should be formulated strictly to avoid the future spread of these multi-drug resistant strains.

6.8 Age and Sex wise distribution in blood culture positive cases:

The current study shows among 26 positive blood culture cases, male neonates were 57.7% cases (15%) and female neonates were 42.3%. Similar studies by Rekha Sriram *et al*, Kuruvilla *et al* and Shrestha *et al* also showed that male neonates were predominance in culture positive cases. This may be attributed to single X chromosome of male neonates increasing susceptibility to infection^{14, 23,52}

Out of 26 blood culture positive cases, 15 cases were in the age group of 0-3 days and it contributes 57.7% of total culture positive cases. Remaining 11 cases were in the age group of 4-28 days and it contributes 42.3% of total blood culture positive cases. Sucila Thangam *et al* also reported in a similar study where early onset neonatal sepsis was more prevalent than late onset neonatal sepsis.¹⁶

6.9 Birth wise distribution in blood culture positive cases:

The present study shows that out of 26 blood culture positive cases, 20 cases (76.7%) were preterm babies and remaining only 6 cases (23.30) were term babies. In similar study by Neema Kayange *et al* , Rabindra N Misra *et al* and Subhranshu Sekhar Kar *et al* observed increased prevalence of neonatal sepsis among low birth weight neonates when compared with normal birth weight neonates.^{17,19,51}

6.10 Gestational age wise distribution in blood culture positive cases:

In the current study out of 26 blood culture positive cases, 23 (86.5%) blood culture positive cases were low birth weight (less than 2500 gm) neonates. Only 3 cases (11.5%) were having birth weight above 2500 gms. In similar study done by Boma *et al*, Bambala Puthattayil Zakariya *et al*, Shrestha *et al* and Kuruvilla *et al* observed increased prevalence of neonatal sepsis among preterm neonates when compared with term neonates. The immuno compromised state of low birth weight and preterm babies may be the cause for more infection in these group.^{23,28,52,88}

6.11.1. Evaluation of C-reactive protein against blood culture positive:

In the present study, the sensitivity of CRP for proven sepsis (more than 6 mg/ L) was 53.84%, its specificity was 64.86%, its positive predictive value was 35.0% and its negative predictive value was 80%.

In a similar study by Sucila Thangam *et al*, during April 2010- September 2010 showed that sensitivity of CRP were 50%, its specificity was 69.4%, its positive predictive value was 38.8% and its negative predictive value was 78.1%.¹⁶ Another similar study done by Benitz MD *et al* from Stanford University of Medicine showed that 54.6% of sensitivity on proven neonatal sepsis and 65.5% of specificity in probable neonatal sepsis among 1002 infants.⁶⁶

Study by Laborada G *et al*, in 48 confirmed blood culture positive cases among 105 suspected neonatal sepsis cases studied, it had been reported that sensitivity of CRP was 69%, Specificity was 96%, positive predictive value was 93% and negative predictive value was 80%.⁶⁷ Another study by Doeller H *et al*, reported that CRP sensitivity 63%, specificity 97%, positive predictive value 83% and negative predictive value 91%.⁶⁸ A study by Kohli-Kochhar R *et al*, the sensitivity, specificity, positive predictive value and negative predictive value of serial CRP measurements were found to be 74.0%, 74.1%, 68.0% and 79.0% respectively in the diagnosis of neonatal sepsis using blood culture as the gold standard.⁷⁵ These three studies showed that slightly higher sensitivity compared to the current study.

A study done by Franz A R *et al* found that 28% of CRP sensitivity, 97% of specificity, 81% of positive predictive value and 77% of negative predictive value.⁶⁹ This study showed lower sensitivity compared to the present study.

FloraChacha *et al*, in their study, showed that the sensitivity and the specificity were 90% and 75% respectively.¹⁵ A study by Nuntnarumit P *et al* found that sensitivity, specificity, positive predictive value and negative predictive value of CRP were 100%, 94%, 91% and 100% respectively.⁷³ Another study done by Hofer N *et al*, showed that CRP had 95% sensitivity and negative predictive value 98% of in the diagnosis of sepsis.⁷⁶ These three studies showed that high sensitivity of CRP compared to the present study.

From these studies, CRP has wide range of sensitivity from 28% to 100%. This may be due to different test methodologies and different reference values. Another reasons for low sensitivity and specificity in some studies may be due to conventional blood culture methods used which has inturn low sensitivity compared to automated system.¹⁵

6.11.2 CRP semi quantitative Assay

The present study shows, among 40 CRP positive cases, 7 cases were found to be with serum CRP level more than 48mg/L category, 5 cases were with CRP level in between 24 - 48mg/L category, one case was with CRP level in between 12-24mg/L category and 26 cases were of CRP level under 6 -12 mg/L category.

Out of 40 CRP positive cases, 14 cases were blood culture positive. Among 14 culture positive cases, 13 cases were due to gram negative organisms (high titre of serum CRP more than 12 mg/L) and one case was due to gram positive organism(low titre of serum CRP more than 6 mg/L). Nora Hofer *et al* also found that increased CRP level in gram negative bacterial infection compared to gram positive bacterial infection.⁷⁶

6.12 Evaluation of Interleukin-6 against blood culture positive:

IL-6 Quantitative Assay

The current study shows, out of 39 IL-6 positive cases, 20 cases were of high positive category (IL-6 level 100-200pg/ml, OD value 1.21-2.34), 5 cases were of moderately positive category (IL-6 level 50-100pg/ml, OD value 0.76-1.20) and 14 cases were of weakly positive category (IL-6 level 6.25 -50 pg/ml, OD value 0.15-0.75).

Among 39 IL-6 positive cases, 25 cases were culture positive (22 cases due to gram negative organisms and 3 cases due to gram positive organism). Among 22 gram negative neonatal cases, 20 cases had high titre of IL-6 and remaining 2 gram negative neonatal sepsis cases had moderately positive titre of IL-6. All the three gram positive neonatal sepsis cases had moderately positive titre of IL-6.

This finding was similar to the study of Laura LR *et al* which revealed Serum of gram negative septicemic cases had increased titre of serum IL-6 level when compared with gram positive septicemic cases. Gram negative bacterial endotoxins are potent inducer for cytokines production by leucocytes comparing with gram positive bacteria.⁹¹

In the current study, the sensitivity of IL-6 for proven sepsis was 96.15%, its specificity was 81.08%, its positive predictive value was 64.10% and its negative predictive value was 98.36%.

Shalini Tripathi *et al* investigated the role IL-6 in the diagnosis of neonatal sepsis and its correlation with the CRP. The sensitivity, specificity, positive predictive value and negative predictive value of IL-6 were 98.4%, 81.2%, 63.5% and 93.5% and those of CRP were 41%, 91%, 87% and 78% respectively.⁸³

Similar result were seen with study done by Emine Kocabas *et al* from Istanbul University, Turkey showed that IL-6 had 96.2% of sensitivity, 89.7% specificity, 86.2% of positive predictive value and 96.1% of negative predictive value.⁸⁴

Mehmet *et al* showed that in 31 neonates with confirmed sepsis among 76 neonates studied, the sensitivity, specificity, positive predictive value and negative predictive value for IL-6 were 100%, 84.2%, 100% and 81.2%.⁸⁵

Ng PC *et al* in their study showed that IL-6 had sensitivity of 89% and 91% of negative predictive value for detecting neonatal infection among 101 clinically suspected sepsis cases (46 culture proven cases). This is relatively in accordance with present study.⁸⁷

A recent study (2013) from Iran, done by Hassan Boskabadi *et al* found that IL-6 had 92.5% of sensitivity, 96.6% of specificity, 97% of positive predictive value and 93% of negative predictive value. The above study shows sensitivity similar to the present study.⁸⁶

The current study confirmed the findings of various authors that IL-6 was more sensitive than CRP in detection of neonatal sepsis. In neonatal sepsis, serum IL-6 level rose earlier than the CRP level.⁷ So serum IL-6 level detection is mainly useful in early detection of neonatal sepsis. Detection of serum IL-6 level also detects the severity of infection and evaluation of the response to antibiotic treatment.

Earlier diagnosis of neonatal sepsis by IL-6 evaluation helps to prevent neonatal mortality and morbidity and avoid unnecessary initiation of empirical antibiotics which in turn helps in preventing drug resistance.

7. SUMMARY

The present study aimed at detecting the bacteriological profile in neonatal sepsis and antibiotic susceptibility pattern of isolates in 100 clinically suspected cases and also detect the role of CRP and IL-6 in the diagnosis of neonatal sepsis.

- Out of 100 clinically suspected neonatal sepsis cases, 58 were male babies and 42 were female babies. Male and Female ratio was 1.38 : 1.
- Out of 100 cases, Early Onset Neonatal Sepsis was 58 and Late Onset Neonatal sepsis was 42.
- Among 58 cases of EONS, 34 cases (58.6%) have normal birth weight and 24 cases (41.4%) have low birth weight.
- Among 42 cases of LONS, 6 cases (14.3%) have normal birth weight and 36 cases (85.7%) have low birth weight.
- Among 58 cases of EONS, 17 cases (29.3%) were preterm babies and 41 cases (70.7%) were term babies.
- Among 42 cases of LONS, 29 cases (69.1%) were preterm babies and 13 cases (30.9%) were term babies.
- Among 58 cases of EONS, 23cases (39.6%) were delivered normally, 27 cases delivered by LSCS and 8 cases (13.8%) were delivered by assisted vaginal delivery.
- Among 42 cases of LONS, 27 cases (64.3%) were delivered normally and 15 cases (35.7%) were delivered by LSCS.

- Out of 100 study cases, 26 cases were blood culture positive.
- Among 26 clinical isolates, *Klebsiella pneumoniae* were 9, *Escherichia coli* were 6, *Pseudomonas aeruginosa* were 4, *Klebsiella oxytoca* were 3 and *Staphylococcus aureus* were 4 isolates.
- Among 12 isolates of *Klebsiella spp*, 10 isolates (83%) were resistant to third generation cephalosporin with ESBL phenotype.
- Out of 26 bacterial isolates, 21 isolates (80.8%) were sensitive to amikacin and 20 isolates (76.9%) were sensitive to gentamicin.
- All 22 isolates of gram negative isolates were sensitive to imipenem.
- Among four *Staphylococcus aureus*, 3 isolates (75%) were methicillin resistant strain (MRSA). But all four isolates were sensitive to vancomycin.
- Mortality rate among study group was 13%.
- Male neonates preponderance (57.7%) were present among 26 culture positive cases .
- Among 26 culture positive cases, 15 cases (57.7%) and 11 cases (42.3%) were EONS and LONS category respectively.
- 20 cases (76.7%) were preterm and 6 cases (23.3%) were term in culture positive cases.
- 23 cases (86.5%) were low birth weight babies and 3cases (11.5%) were normal birth weight babies in culture positive cases.

- 61.54% of neonates were delivered by normally, 30.77% of were delivered by LSCS and 7.69% were delivered by assisted vaginal delivery.
- Serum CRP level detected by latex agglutination method had 53.84% of sensitivity and 64.86% of specificity against blood culture as a reference test.
- Positive predictive value and negative predictive value of CRP were 35% and 80% respectively.
- Serum IL-6 level detected by ELISA had sensitivity of 96.15% and specificity of 81.08% against blood culture as a reference test.
- Positive predictive value and negative predictive value of IL-6 were 64.1% and 98.36% respectively.

8. CONCLUSION

- *Klebsiella spp* were the most common organisms for causing both EONS and LONS in the present study.
- The findings of the present study confirm that the serum level of IL-6 is a more reliable marker than the serum levels of CRP.
- Serum level of IL-6 is also useful in evaluation of response of neonatal sepsis to antibiotic therapy.
- The benefit of measuring serum IL-6 routinely in the diagnosis and follow up of neonatal sepsis, is that it reduces neonatal mortality and morbidity and also reduces the hospital stay and cost of health care.
- Neonatal sepsis can be prevented by proper hand washing, proper antenatal care, proper aseptic clean labour ward and proper aseptic clean NICU.
- To strictly adhere to institutional infection control committee's protocol for preventing multi drug resistant organism causing neonatal sepsis.
- Low birth weight neonates need more care for preventing nosocomial infection by multi drug resistant organisms.

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ANNEXURE – 1

1. Nutrient agar medium:

Composition

Ingredients gram/liter

Peptic digest of Animal Tissue 5.00

Sodium Chloride 5.00

Beef Extract 1.50

Yeast Extract 1.50

Agar 15.00

Twenty-eight grams of dehydrated nutrient agar medium was added to 1000 ml of cold distilled water in a flask and boiled to dissolve the medium completely. The medium was then sterilized in an autoclave at 121°C and 15 lbs pressure for 15 minutes. The sterile media were stored in a refrigerator at 4°C for future use.

2. MacConkey agar medium:

Composition - Ingredients gram/liter

Peptone 19.0

Lactose 10.0

NaCl 5.0

Na- Deoxycholate 1.0

Neutral Red 0.03

Crystal Violet 0.001

Agar 15.0

Fifty-two grams of dehydrated MacConkey agar medium was suspended in 1000 ml of cold distilled water and boiled to dissolve the medium completely. The solution was then sterilized by autoclaving at 121⁰C and 15 lbs pressure for 15 minutes.

3. Blood agar medium

Composition

Ingredients gram/liter

Heart infusion 500.00

Tryptose 10.00

Sodium chloride 5.00

Agar 15.00

Forty grams of the dehydrated blood agar medium was suspended in 1000 ml cold distilled water in a flask and boiled to dissolve the medium completely. It was then sterilized by autoclaving at 121⁰C and 15 lbs pressure for 15 minutes. The autoclaved materials were allowed to cool to a temperature of 45⁰C in a water bath. Defibrinated 5-10% sheep blood was then added to the medium aseptically and distributed to sterile petri dishes. Sterile media was stored in refrigerator at 4⁰C for future use.

4. Muller Hinton agar medium

Composition

Ingredients gram/liter

Beef dehydrated infusion 300

Casein hydrolysate 17.50

Starch agar 17.00

Agar 17.00

Thirty-eight grams of dehydrated Mueller Hinton agar medium was suspended in 1000 ml cold distilled water and boiled to dissolve the medium completely. The solution was then sterilized by autoclaving at 121°C and 15 lbs pressure for 15 minutes. The autoclaved media was stored in the refrigerator and used later.

5. McFarland Standard (0.5):

Reagents:

Sulphuric acid, 1%: To 100 ml of distilled water, 1 ml of conc.sulphuric acid is added. Barium chloride, 1.175%: To 100 ml of distilled water, 1.175gm of barium chloride is added and mixed well.

To prepare McFarland 0.5 standards:

To 85 ml of 1% conc.sulphuric acid, 0.5 ml of Barium chloride is added in a flask while constantly swirling the flask. Bring to 100 ml with 1% conc.sulphuric acid. Aliquot in test tubes and cap tubes tightly. Store in the dark at room temperature for 3 months or longer.

ANNEXURE – 2

Role of C-reactive protein and interleukin-6 in the diagnosis of neonatal sepsis

PROFORMA

Name : B/O Mrs. : IP No.:

Age : days

Sex :

Gestational age : in weeks Preterm / Term

Nature of Delivery : Normal/Assisted/LSCS

Birth weight :

Clinical diagnosis :

Investigation

Lab. No. :

Blood culture Result :

Antibiotics Sensitive to :

Antibiotics Resistance to :

CRP Result : Titre :

IL-6 Result : O.D Value:

Outcome : Recovery and discharge/ death

MASTER CHART

S.No	Age (in days)	Sex	Gestational Age (in weeks)	Mode of delivery	Birth weight (gms)	Blood Culture	Bacterial isolates	Gentamicin	Amikacin	Cefotaxime	Ceftriaxone	Ceftazidime	Ceftazidime and Clav	Cotrimoxazole	ciprofloxacin	Imipenem	Cefperazone-Sulbactam	Ampicillin	Cefoxitin	Erythromycin	Clindamycin	Vancomycin	CRP	CRP Titre (mg/L)	IL-6	OD Value	Out come
1	1	Female	Preterm	Normal	2100	N																	P	>6	N	0.1	Cured
2	3	Male	Preterm	LSCS	1950	N																	P	>6	N	0.14	Cured
3	3	Male	Preterm	Normal	1900	N																	P	>6	N	0.08	Cured
4	1	Female	Preterm	LSCS	2000	P	<i>K.pneumoniae</i>	S	S	S	S	S	S	S	S	S	S						P	> 48	P	2.24	Cured
5	4	Female	Term	LSCS	3200	N																	N		N	0.13	Cured
6	2	Female	Term	LSCS	2800	P	<i>S.aureus</i>	S	S					S	S			S	S	S	S	S	P	>6	P	1.1	Cured
7	4	Male	Term	Normal	3000	N																	P	>6	P	0.22	Cured
8	4	Male	Term	LSCS	1900	N																	N		N	0.08	Cured
9	2	Female	Term	LSCS	3100	N																	N		N	0.11	Cured
10	5	Female	Preterm	Normal	1900	N																	P	>6	N	0.14	Cured
11	4	Male	Term	LSCS	1600	N																	P	>6	N	0.07	Cured
12	6	Female	Preterm	LSCS	1700	N																	N		N	0.09	Cured
13	2	Male	Term	Assisted	3800	N																	N		N	0.1	Cured
14	2	Male	Term	Normal	1800	P	<i>K.pneumoniae</i>	S	S	R	R	R	S	R	S	S	S						P	> 48	P	1.86	Death
15	4	Female	Term	Normal	3400	N																	P	>6	P	0.35	Cured
16	2	Female	Term	LSCS	3900	N																	N		N	0.13	Cured
17	6	Male	Preterm	Normal	1700	P	<i>E.coli</i>	R	R	R	R	R	S	R	R	S	S						P	> 48	P	1.96	Death
18	4	Male	Term	LSCS	1700	N																	P	>6	N	0.14	Cured
19	4	Male	Preterm	LSCS	1600	N																	P	>6	N	0.07	Cured
20	3	Male	Term	Normal	2600	N																	N		N	0.13	Cured
21	3	Female	Preterm	Normal	2200	N																	P	>6	P	0.48	Cured
22	2	Male	Term	LSCS	3500	N																	P	>6	N	0.14	Cured
23	3	Male	Term	Assisted	3500	N																	P	>6	P	0.61	Cured
24	2	Male	Term	Assisted	3300	N																	N		N	0.08	Cured
25	1	Female	Term	LSCS	2900	N																	N		N	0.09	Cured
26	2	Female	Preterm	Normal	2100	N																	N		N	0.1	Death
27	3	Male	Term	LSCS	2400	N																	N		N	0.11	Cured
28	2	Female	Term	Normal	2400	N																	N		N	0.13	Cured
29	8	Male	Preterm	Normal	2200	N																	N		N	0.09	Cured
30	5	Male	Preterm	Normal	2000	N																	N		N	0.07	Cured

31	3	Female	Term	LSCS	2400	P	<i>P.aeruginosa</i>	R	R	R	R	R	S	R	R	S	S						P	>24	P	2.22	Death
32	2	Male	Preterm	LSCS	2000	P	<i>K.pneumoniae</i>	S	S	R	R	R	S	S	S	S	S						P	> 48	P	2.34	Death
33	7	Male	Preterm	LSCS	2100	P	<i>K.pneumoniae</i>	S	S	R	R	R	S	S	S	S	S						P	> 48	P	1.59	Death
34	6	Male	Term	Normal	2600	N																N		N	0.12	Cured	
35	3	Male	Preterm	Normal	1900	P	E.coli	S	S	S	S	S	S	S	S	S	S						P	>12	P	2.32	Cured
36	3	Female	Preterm	Normal	2500	N																N		N	0.13	Cured	
37	5	Male	Term	LSCS	2600	N																N		N	0.07	Cured	
38	3	Female	Term	LSCS	2700	N																P	>6	P	0.54	Cured	
39	3	Male	Term	LSCS	2650	N																P	>6	N	0.08	Cured	
40	2	Male	Term	Assisted	2700	N																N		N	0.13	Cured	
41	3	Male	Term	Assisted	2900	P	<i>S.aureus</i>	S	S					S	S			S	S	S	S	S	N		P	0.82	Cured
42	5	Male	Term	Normal	2400	N												S	S	S	S	S	P	>6	N	0.07	Cured
43	3	Female	Preterm	Normal	2200	N																N		N	0.12	Cured	
44	3	Male	Preterm	Normal	2000	P	<i>P.aeruginosa</i>	R	S	R	R	R	S	R	S	S	S						N		P	1.76	Death
45	7	Male	Term	LSCS	3500	N																N		N	0.08	Cured	
46	1	Male	Term	Assisted	3400	N																P	>6	P	0.62	Cured	
47	8	Female	Preterm	Normal	1800	P	<i>K.pneumoniae</i>	S	S	R	R	R	S	R	S	S	S						P	>24	P	2.06	Death
48	3	Male	Term	LSCS	2400	N																N		N	0.09	Cured	
49	4	Female	Term	Normal	2300	N																N		N	0.14	Cured	
50	3	Female	Term	Normal	2000	N																P	>6	N	0.14	Death	
51	2	Male	Term	LSCS	2800	N																N		N	0.13	Cured	
52	22	Female	Preterm	LSCS	1700	P	<i>K.oxytoca</i>	S	S	S	S	S	S	S	S	S	S						N		P	1.34	Death
53	1	Male	Term	Assisted	3300	P	<i>S.aureus</i>	S	S					R	S			R	R	S	S	S	N		N	0.07	Death
54	12	Female	Preterm	LSCS	1900	N																N		N	0.07	Cured	
55	1	Male	Term	LSCS	3200	N																P	>6	N	0.08	Cured	
56	3	Male	Preterm	Normal	2400	P	<i>K.pneumoniae</i>	S	S	R	R	R	S	R	R	R	S						P	>24	P	1.51	Cured
57	24	Female	Preterm	Normal	1900	P	<i>K.pneumoniae</i>	R	R	R	R	R	S	R	R	S	S						P	> 48	P	1.25	Cured
58	13	Male	Preterm	Normal	2200	N																P	>6	N	0.09	Cured	
59	3	Female	Term	LSCS	3200	N																P	>6	N	0.12	Cured	
60	3	Female	Preterm	Normal	2100	P	<i>E.coli</i>	S	S	S	S	S	S	S	S	S	S						P	>24	P	2.34	Cured
61	6	Female	Preterm	Normal	2400	N																N		N	0.12	Cured	
62	6	Male	Term	LSCS	2400	N																N		N	0.14	Cured	
63	3	Male	Term	LSCS	2700	N																N		N	0.1	Cured	
64	4	Male	Preterm	Normal	2300	N																N		N	0.1	Cured	
65	7	Male	Preterm	Normal	2100	P	<i>S.aureus</i>	S	S					R	S			R	R	R	R	S	N		P	0.97	Cured
66	2	Male	Term	LSCS	2800	N																P	>6	P	0.59	Cured	
67	4	Female	Preterm	Normal	2200	P	<i>P.aeruginosa</i>	S	S	R	R	R	S	R	S	S	S						N		P	0.98	Cured
68	3	Female	Term	LSCS	2700	N																N		N	0.12	Cured	
69	4	Male	Preterm	Normal	2300	N																N		P	0.24	Death	
70	3	Male	Preterm	Normal	2100	P	<i>K.pneumoniae</i>	R	R	R	R	R	S	S	S	S	S						N		P	1.18	Cured
71	2	Female	Term	Assisted	3000	N																N		N	0.13	Cured	
72	2	Female	Term	LSCS	2900	N																P	>6	P	0.38	Death	
73	4	Male	Preterm	Normal	2100	P	<i>E.coli</i>	S	S	S	S	S	S	S	S	S	S						N		P	1.83	Cured
74	4	Male	Preterm	Normal	2200	N																N		N	0.07	Cured	
75	17	Female	Preterm	Normal	2000	N																N		P	0.66	Cured	
76	4	Female	Term	LSCS	2100	N																N		N	0.07	Cured	
77	10	Male	Preterm	Normal	2400	N																N		N	0.14	Cured	
78	3	Male	Term	LSCS	2300	N																P	>6	P	0.21	Cured	
79	2	Female	Term	Normal	2700	N																N		N	0.13	Cured	
80	3	Female	Term	Normal	2800	N																N		N	0.13	Cured	

81	5	Male	Preterm	LSCS	2400	N																N		N	0.14	Cured
82	2	Male	Preterm	Normal	2000	P	<i>K.pneumoniae</i>	S	S	S	S	S	S	S	S	S						N		P	2.21	Cured
83	3	Male	Term	LSCS	2900	N																P	>6	P	0.32	Cured
84	1	Male	Term	Normal	3100	N																N		N	0.07	Cured
85	1	Female	Term	Normal	2500	N																N		P	0.43	Cured
86	2	Female	Term	LSCS	2400	P	<i>E.coli</i>	S	S	S	S	S	S	S	S	S						P	> 48	P	1.44	Cured
87	2	Female	Preterm	Normal	1900	N																N		N	0.08	Cured
88	4	Male	Preterm	Normal	2100	N																N		N	0.07	Cured
89	3	Male	Term	LSCS	2600	N																P	>6	N	0.1	Cured
90	3	Female	Preterm	Normal	2200	P	<i>P.aeruginosa</i>	S	S	S	S	S	S	R	S	S	S					N		P	1.33	Cured
91	4	Male	Preterm	Normal	2000	P	<i>E.coli</i>	S	S	S	S	S	S	R	S	S	S					P	>24	P	1.93	Cured
92	5	Female	Preterm	LSCS	2200	P	<i>K.oxytoca</i>	S	S	R	R	R	S	R	S	S	S					N		P	1.28	Cured
93	4	Male	Preterm	Normal	1900	P	<i>K.oxytoca</i>	R	R	R	R	R	S	R	R	S	S					N		P	1.51	Cured
94	1	Female	Term	LSCS	2900	N																P	>6	N	0.09	Cured
95	2	Female	Term	LSCS	3000	N																N		N	0.09	Cured
96	6	Male	Preterm	Normal	2200	N																N		N	0.07	Cured
97	4	Male	Preterm	Normal	2100	N																N		P	0.54	Cured
98	3	Male	Term	Normal	3100	N																N		N	0.13	Cured
99	4	Male	Preterm	Normal	2400	N																N		N	0.14	Cured
100	2	Female	Term	LSCS	2600	N																N		N	0.09	Cured